CE LIBRARY
133
NOV 2 1934

VOLUME 24 NUMBER 10

OCTOBER, 1934

Phytopathology

An International Journal

Official Organ of

The American Phytopathological Society

CONTENTS

Chemical studies on the virus of tobacco mosaic I. Some effects of trypsin ..... W. M. STANLEY 1055

The wedge graft as a means of controlling overgrowths at the union of nursery apple trees ..... R. F. SUIT 1086

Conidial production in species of Cercospora in pure culture. CLATUS M. NAGEL 1101

Application of stream double refraction in the identification of streak diseases of tomato. WILLIAM N. TAKAHASHI and T. E. RAWLINS 1111

Phytopathological Notes 1116

Cytological changes in the callus of the graft union in connection with curly top in tomatoes. J. DUFRÉNOY and MICHAEL SHAPOVALOV

A fungus gall on Viburnum mistaken for crown gall. NELLIE A. BROWN

White pycnia and aecia of Puccinia graminis. RALPH U. COTTER

Mercury ammonium silicate as a Gladiolus corm treatment. RICHARD P. WHITE

The effect of mosaic on bloom production of the Talisman rose. RICHARD P. WHITE

Increase of tobacco-mosaic virus in the absence of chlorophyll and light ..... FRANCIS O. HOLMES

Aphid transmission of potato yellow dwarf ..... KARL KOCH

Medium for growth of pythiaceous fungi ..... F. P. MEHRLICH

Book Reviews 1129

Report of the eighteenth annual meeting of the Pacific Division of The American Phytopathological Society 1134

Price { $6.00 per year, United States and dependencies,
$6.25 per year, Canada
$6.50 per year, elsewhere

PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

COR. LIME AND GREEN STREETS, LANCASTER, PA.

Entered at the post-office in Lancaster, Pa., as second-class matter.

DIRECTORY OF ADVERTISERS

AMERICAN CYANAMID CO., 535 Fifth Ave., New York, N. Y.	AERO CYANAMID FOR SOIL STERILIZATION AND WEED CONTROL.
BAYER–SEMESAN CO., INC., DuPont Building, Wilmington, Del.	SEED AND SOIL TREATMENTS. NEW IMPROVED CERESAN. NEW IMPROVED SEMESAN JR.
CORNING GLASS WORKS, Corning, New York.	PYREX CYLINDRICAL JARS.
CLAY–ADAMS CO., 25 East 26th St., New York, N. Y.	SLIDES AND COVER GLASSES. MICROSCOPIC PROJECTION AND DRAWING APPARATUS.
DIFCO LABORATORIES, 920 Henry St., Detroit, Mich.	DEHYDRATED CULTURE MEDIA.
THE GRASSELLI CHEMICAL CO., Cleveland, Ohio.	SPRAY MATERIALS AND DUSTS.
HELMINTHOLOGICAL SOCIETY OF WASHINGTON, J. R. Christe, Editor. U. S. Bureau of Plant Industry, Washington, D. C.	A NEW JOURNAL DEVOTED TO HELMINTHOLOGY, PROTOZOOLOGY, AND RELATED FIELDS.
LINDER & COMPANY, INC., 298 North Beacon St., Brighton Station, Boston, Mass.	VAPORIZING SULFUR. SULFUR-NEBULATOR.
MALLINCKRODT CHEMICAL WORKS, St. Louis, Montreal, Philadelphia, New York.	FUNGINOX, CURE-DAMP, CALOGREEN.
MERCK & CO., INC., Rahway, New Jersey.	COPPER OXIDE RED POWDER, CALOMEL, R. T. 50.
N. V. POTASH EXPORT M. Y., INC., 19 West 44th St., New York, N. Y.	AGRICULTURAL AND SCIENTIFIC BUREAU. POTASH FERTILIZER.
NIAGARA SPRAYER AND CHEMICAL CO., INC., Middleport, New York.	DUSTERS, DUSTS, AND SPRAYS. KOLOFOG, POMO-GREEN.
ROHM & HAAS & CO., INC., 222 W. Washington Square, Philadelphia, Pa.	RED COPPER OXIDE. FORM-O-FUME FORMALDEHYDE DUST.
THE ROESSLER & HASSLACHER CHEMICALS DEPT., E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.	P. A. C. FORMALDEHYDE.
SCIENCE PRESS, Lime and Green Sts., Lancaster, Pa.	PRINTERS OF SCIENTIFIC JOURNALS, BOOKS AND MONOGRAPHS.
TRIARCH BOTANICAL PRODUCTS, Geo. H. Conant, Ripon, Wis.	PRESERVED PLANTS. MICROSCOPE SLIDES.
PHYTOPATHOLOGICAL CLASSICS, H. H. Whetzel Mgr., Cornell University, Ithaca, N. Y.	NUMBERS 1, 2, AND 3 PHYTOPATHOLOGICAL CLASSICS.
WARREN–KNIGHT CO., 136 N. 12th St., Philadelphia, Pa.	WRICO LETTERING GUIDES AND DRAFTING SUPPLIES.

PHYTOPATHOLOGY

An International Journal
Official Organ of The American Phytopathological Society

EDITORS

H. B. HUMPHREY, Editor in Chief, U. S. Department of Agriculture, Washington, D. C.
H. M. QUANJER, Editor for Europe, Instituut voor Phytopathologie, Wageningen, The Netherlands.
ANNIE RATHBUN GRAVATT, U. S. Department of Agriculture, Washington, D. C.
EUBANKS CARSNER, Citrus Experiment Station, Riverside, Calif.

ASSOCIATE EDITORS

J. C. GILMAN, Iowa State College, Ames, Iowa.
W. D. VALLEAU, University of Kentucky, Lexington, Ky.
W. F. HANNA, Manitoba Agricultural College, Winnipeg, Canada.
F. L. DRAYTON, Central Experimental Farm, Ottawa, Canada.
JESSIE I. WOOD, U. S. Department of Agriculture, Washington, D. C.
B. F. LUTMAN, University of Vermont, Burlington, Vt.
LEE M. HUTCHINS, Fort Valley, Ga.
C. D. LA RUE, University of Michigan, Ann Arbor, Mich.
GLENN G. HAHN, Yale University, New Haven, Conn.
R. P. WHITE, State College of Agriculture and Mechanic Arts, New Brunswick, N. J.
F. O. HOLMES, The Rockefeller Institute for Medical Research, Princeton, N. J.
J. J. CHRISTENSEN, University Farm, St. Paul, Minn.

BUSINESS MANAGEMENT

F. C. MEIER, Business Manager, U. S. Department of Agriculture, Washington, D. C.
R. S. KIRBY, Advertising Manager, Pennsylvania State College, State College, Pa.

NOTICES

Subscription price. $6.00 per year United States and dependencies; Canada $6.25; other countries $6.50; current single numbers 60 cents. The journal is issued monthly beginning with Volume VIII, January, 1918.

Advertisements. Rates may be secured from business management. No indorsement of any statement of claims made in advertisements is assumed by this journal or by The American Phytopathological Society.

Requests to supply copies of the journal must be made within 60 days from date of issue. Date of issue January 15 and monthly thereafter.

Separates. No gratis copies are supplied. A printed schedule of prices is submitted with the proof, and authors may secure separates by placing an order on the blanks provided when proof is returned.

Back Volumes IX to XXII inclusive, (except Vol. IX, No. 1, which is not available) unbound at $8.00 per volume. All earlier volumes obtainable only in broken numbers.

Separate Numbers from Volumes IX to XXII, $1.00 per copy; from Volumes I to VIII, $2.00 per copy. The following numbers of Volumes I to VIII, inclusive, are obtainable; Volume I, Nos. 1, 4, 5, 6 and index: Vol. II, Nos. 1, 5, and index: Vol. III, Nos. 4, 5, 6: Vol. VI, index only: Vol. VIII, Nos. 2 and 12 and index.

Manuscript may be sent to the nearest member of the editorial board. Clearness, brevity, and conciseness are essential. In form and style, such as punctuation, spelling

and use of italics manuscripts should conform to the best usage in recent numbers of this journal. Manuscripts should be typed on one side of the paper, double spaced, and sent unfolded. All papers longer than four printed pages should include a summary. The name and address of the institution at which the work was done should be included at the end of the summary. All footnotes should be numbered consecutively with arabic numerals except in tables, where small letters should be used. Each table should have a heading stating clearly and concisely its contents. References to literature should be made as footnotes only when five or fewer citations are given. The simplified method of citing literature used in recent numbers of this journal should be followed. References in "Literature Cited" should be listed alphabetically and numbered consecutively.

Illustrations necessarily must be limited in number, and photographs, to reproduce satisfactorily, must be of the best quality. Authors desiring unusual numbers of illustrations or special types of illustrations will be asked to bear part of the expense. All illustrations are reproduced on the same grade of paper and will be run as text figures wherever possible. Plates should be used only for a number of small illustrations which can not be run conveniently as individual text figures. Individual illustrations within a plate should be designated by letters to avoid confusion with text figures which are designated by arabic numerals. Authors should arrange plates to conform to page size and shape ($4\frac{1}{2}$ inches by 7 inches) after making due allowance for reductions. Specific reference to each illustration must be made in the text. There should be a complete legend for each illustration. All legends should be on separate paper and not on the illustration itself.

The responsibility for statements, whether of fact or opinion, rests entirely with the writers thereof.

The American Phytopathological Society

OFFICERS

President

NEIL E. STEVENS, U. S. Department of Agriculture, Washington, D. C.

Vice-President

G. W. KEITT, University of Wisconsin, Madison, Wis.

Secretary-Treasurer

F. C. MEIER, U. S. Department of Agriculture, Washington, D. C.

Councilors

J. C. ARTHUR, Purdue University, La Fayette, Ind.
F. D. HEALD, State College of Washington, Pullman, Wash.
H. B. HUMPHREY, U. S. Department of Agriculture, Washington, D. C.
CARL HARTLEY, U. S. Department of Agriculture, Washington, D. C.

Councilors Representing Divisions

C. E. OWENS, State College, Corvallis, Oreg. (Western Division).
V. H. YOUNG, University of Arkansas, Fayetteville, Ark. (Southern Division).

PHYTOPATHOLOGY

VOLUME 24 NUMBER 10

OCTOBER, 1934

CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC
I. SOME EFFECTS OF TRYPSIN

W. M. STANLEY

(Accepted for publication July 26, 1934)[1]

INTRODUCTION

There has been little work reported on the action of enzymes on animal and plant viruses, despite the fact that digestion of a virus with a proteolytic enzyme would serve as an indication that viruses are proteins. Vinson and Petre (15) and Lojkin and Vinson (5) reported that emulsin, pepsin, and yeast extract had no effect on the infectivity of tobacco-mosaic virus, but that trypsin and papain inactivated this virus and that the inactivation caused by trypsin was probably due to the hydrolytic action of the enzyme. Somewhat later Matsumoto and Somazawa (6) reported that the infective principle and certain precipitating agents of juice from tobacco plants infected with mosaic withstood the action of rather large amounts of trypsin fairly well, although certain antigenic properties were greatly impaired. Caldwell (1) found that tryspin, when added to aucuba-mosaic virus, caused a loss in infectivity, but that some of the infectivity of the virus returned when the mixture was heated, a result with which Vinson (14) has recently agreed.

If the virus were actually split or digested by trypsin, as first suggested by Vinson, reactivation by heat would be improbable. Furthermore, if the inactivation were due to proteolysis one would expect pepsin, rather than trypsin, to be an active proteolytic agent. Trypsin usually fails to digest native proteins, whereas pepsin is usually able to digest both native and denatured proteins. It was decided, therefore, to reinvestigate the action of trypsin and of pepsin on the virus of tobacco mosaic. The present paper records the results of a study of some effects of trypsin upon this virus.

MATERIALS AND METHODS

Two preparations of tobacco-mosaic virus were used in the experiments to be reported. One consisted of untreated juice from *Nicotiana tabacum*

[1] Published at the expense of The Rockefeller Institute for Medical Research out of the order determined by date of receipt of the manuscript.

L. var. Turkish infected with tobacco mosaic. The other, which was used in most of the experiments, was prepared from infectious juice by a modification of the method of Vinson and Petre (16). Juice from Turkish tobacco plants infected with mosaic was precipitated with lead acetate and eluted with 0.1 M potassium phosphate at pH 7. The volume of this purified preparation was so adjusted that its infectivity was comparable to that of the untreated infectious juice. Dilutions of purified virus were made with 0.1 M phosphate at pH 7 and dilutions of untreated infectious juice were made with distilled water.

The trypsin used was a highly purified crystalline preparation[2] and commercial Fairchild trypsin. It is believed that the crystalline trypsin consists of only one enzyme, whereas commercial trypsin is a mixture of several enzymes (8). Because of this the crystalline trypsin preparation was used in the greater part of the work. It was employed in the form of a solution in 0.01 N hydrochloric acid, usually containing 0.1 mg. trypsin nitrogen per cc. The addition of this solution to the virus preparations caused little or no change in the hydrogen-ion concentration of the latter, since the preparations were well buffered. The powdered commercial trypsin was added directly to the virus preparations. One drop of toluene per 10 cc. of solution was added as a preservative. Controls for the commercial trypsin consisted of virus preparations that had been treated in exactly the same manner as the trypsin-virus solutions, except that no trypsin was added. Controls in the case of the crystalline trypsin consisted of virus preparations that had been treated in exactly the same manner as the trypsin-virus solutions, except that 0.01 N HCl was added instead of the trypsin solution. With the above exceptions, the protein-treated solutions and the corresponding control solutions were prepared, incubated, and inoculated in the same manner and under identical conditions.

The concentration of the virus was estimated by the local-lesion method developed by Holmes (2) and by Price (9) for *Nicotiana glutinosa* L. and *Phaseolus vulgaris* L. variety Early Golden Cluster, respectively. Because of the desirability of measuring as accurately as possible the relative infectivity of the virus in the protein-treated solution and in the control, the half-leaf method (2, 11) of inoculation was used whenever possible. The average number of lesions produced when the preparation of virus and protein was inoculated on one-half of the leaf was compared with the average produced by the control virus preparation on the other half of the same leaf. The diminution of the infectivity of a given virus sample by means of extraneous materials or by means of a change in hydrogen-ion

[2] The writer is indebted to Dr. J. H. Northrop, Dr. M. Kunitz and Dr. M. L. Anson for the purified proteins used in this investigation.

concentration, as well as the opposite effect, consisting of the enhancement of the infectivity of a given virus preparation by similar means, need always be taken into account whenever the relative amount of virus in a sample is under consideration. It was found, for example, that at some hydrogen-ion concentrations, and in the presence of certain materials, some susceptible plants failed to produce lesions when inoculated with virus preparations, even though virus was present in an active condition and in high concentration, as was demonstrated by inoculation of other species of test plants. Therefore, virus preparations which gave negative results on one plant species were tested at a favorable hydrogen-ion concentration on a number of different species of plants before the absence of virus infectivity was accepted. Even then it is possible, of course, that virus may be present, but that it fails to infect the plants.

In most of the experiments the same virus preparation and its control were tested on 6 or more plants of *Phaseolus vulgaris* having 12 or more primary leaves and on 1 or 2 plants of *Nicotiana glutinosa* having 5 leaves each. Inoculation was accomplished by rubbing the upper surface of one-half of each leaf with a small piece of folded bandage gauze moistened with the test solution. The other half of the same leaf was similarly treated with the control solution and the leaf was then washed in running tap water. The leaf was held under the stream of water so that the wash water ran from the control half towards the protein-treated half and not in the reverse direction. This was done to prevent trypsin in the wash water from coming in contact with the control half of the leaf. In certain instances the tests were made both by the half-leaf and whole-leaf methods to detect possible errors from this source. The lesions, which usually appeared within 48 hours after inoculation, were counted at the end of 4 or 5 days. Unless otherwise specified, all inoculations in the following experiments were made by this rubbing method.

EXPERIMENTAL

Studies on the Proteolytic Action of Trypsin

Comparison of the Effects of Trypsin and Trypsinogen. Table 1 gives the results of a series of experiments designed to demonstrate, under various conditions, the effect of crystalline trypsin and crystalline trypsinogen on the infectivity of a purified preparation of tobacco-mosaic virus. Trypsinogen, which is trypsin before it has been activated, has no proteolytic activity. In this experiment, in which plants of *Nicotiana tabacum* were also used as test plants, the inoculation of these plants was made by puncturing 1 leaf of each plant with 5 pins that had been dipped in the test solution. The inoculation of *N. glutinosa* and *Phaseolus vulgaris* was

TABLE 1.—*Comparison of the effects of trypsin and trypsinogen on the infectivity of a purified preparation of tobacco-mosaic virus.*

Dilution →	Tested		Undiluted			1–10		1–100		1–1000
Tested on →	↓		P. vulgaris	N. glutinosa	Turkish % takes	P. vulgaris	N. glutinosa	P. vulgaris	N. glutinosa	P. vulgaris
Virus pH 7.0	At once	L_1	142.0[a]	100.0[b]		77.5	36.2	49.0	3.26	5.20
		R_2	84.0	101.0	39.5[c]	59.4	24.5	42.6	23.1	1.94
						24.0	31.8			
		Av.	113.0	100.5		53.6	30.8	45.8	13.2	3.5
	After 6 days	L_4	51.2	81.6		13.7	40.1	3.72	6.4	2.56
		R_5	32.2	84.0	10.0	10.6	69.0	5.30	3.6	2.75
						6.7				
		Av.	41.7	82.8		10.3	54.5	4.5	5.0	2.6
Heated virus 70° C.	At once	L_7	7.9	18.1		16.1	4.4	0.16		
		R_8	28.7	26.4		19.6	22.6	0.00		
						2.2	8.7			
		Av.	18.3	22.2		12.6	11.9	0.08		
	After 6 days	L_{10}	14.7	29.3		7.05	13.3	1.94		
		R_{11}	9.05	14.4	6.0	12.7	15.6	4.75		
						10.3				
		Av.	11.9	21.8		10.0	14.4	3.3		
Trypsin-virus	At[d] once	L_2	0.00	1.40		0.15	1.00	1.24	3.8	0.05
		R_3	0.00	2.74	0.00	0.12	4.13	0.50	1.4	0.33
						0.00	0.90			
		Av.	0.00	2.07		0.1	2.0	0.9	2.6	0.2
	After[e] 6 days	L_5	0.00	4.4		0.00	13.4	0.41	0.13	0.31
		R_6	0.20	10.8	1.0	0.06	11.3	0.00	0.93	0.11
						0.06				
		Av.	0.10	7.6		0.04	12.3	0.2	0.5	0.2
Heated trypsin-virus 70° C.	At[f] once	L_8	0.00	15.7		0.35	3.30	0.00		
		R_9	0.05	12.3		0.22	2.20	0.00		
						0.06	1.90			
		Av.	0.02	14.0		0.2	2.5	0.00		
	After[f] 6 days	L_{11}	3.40	3.54		2.20	4.87	0.53		
		R_{12}	2.75	6.46	3.0	1.94	6.74	1.06		
						2.90				
		Av.	3.10	5.0		2.3	5.8	0.8		

TABLE 1.—(*Continued*)

Dilution →	Tested		Undiluted			1–10		1–100		1–1000
Tested on →	↓		P. vulgaris	N. glutinosa	Turkish % takes	P. vulgaris	N. glutinosa	P. vulgaris	N. glutinosa	P. vulgaris
Trypsinogen-virus	At[f] once	L_3	2.54	0.13		2.44	1.00	0.78	0.80	0.50
		R_1	5.05	0.13	0.0	1.12	1.33	0.15	1.07	0.00
						0.22	0.50			
		Av.	3.8	0.13		1.2	0.9	0.5	0.9	0.25
	After[f] 6 days	L_6	0.13	0.47		0.00	0.33	0.06	0.53	0.05
		R_4	0.28	0.26	0.0	0.44	1.47	0.16	0.80	0.12
						0.22				
		Av.	0.20	0.36		0.2	0.9	0.1	0.7	0.13
Heated trypsinogen-virus 70° C.	At[f] once	L_9	0.19	0.00		0.16	0.10	0.05		
		R_7	2.40	0.00		0.58	0.00	0.11		
						0.28	0.10			
		Av.	1.30	0.00		0.3	0.07	0.08		
	After[f] 6 days	L_{12}	0.12	0.66		0.06	0.80	0.11		
		R_{10}	0.07	0.80	0.0	0.00	0.47	0.06		
						0.16				
		Av.	0.10	0.73		0.07	0.6	0.08		

[a] Numbers represent the average number of lesions on 12 or more half-leaves of *P. vulgaris* var. Early Golden Cluster.
[b] Numbers represent the average number of lesions on 5 or more half-leaves of *N. glutinosa.*
[c] Numbers represent the per cent of infected Turkish tobacco plants out of 50 or 100 inoculated plants.
[d] Tryptic activity unchanged.
[e] Decreased tryptic activity.
[f] No tryptic activity.

by the rubbing method previously described. Since it was desired to find the relation between the infectivity of the untreated virus and the 2 treated virus solutions, as well as the relation between the 2 treated virus solutions, each solution was tested against each of the other 2 solutions on leaf-halves. This was repeated after heating and was also repeated at the end of 6 days, both before and after heating. The designations in column 3 refer to the leaf-half on which the various solutions were tested and the average of such tests. For example, the first horizontal row of numbers designated by L_1 represents the average number of lesions per leaf produced on the left sides of the leaves by various concentrations of the virus, and the row of numbers designated by R_1 represents the average number of lesions produced on the right sides of these same leaves by the trypsinogen-virus solution. Likewise, R_2 represents the average number of lesions caused by the same virus preparation on the right sides of the leaves, and L_2, the average number of lesions caused by the trypsin-virus solution on the left sides of the same leaves. Each solution was tested twice, once on the right halves of one set of leaves and once on the left halves of another set of leaves. A third test was included with all solutions at a 1–10 dilution. The horizontal rows, marked "av.," represent the average of all tests of a given solution at a given dilution and are included in order that a rapid comparison of any two solutions may be made.

The results given in table 1 show that the reduction in infectivity of the virus by both trypsin and trypsinogen took place immediately. The infectivity of the virus-trypsin mixture increased on incubation for 6 days at 37° C., or on heating for 20 minutes at 70° C. It also increased on dilution, more lesions being produced when the virus-trypsin solution was diluted 1–100 than when used undiluted. The increase in infectivity by heating the virus-trypsin solution is similar to the results obtained by Caldwell (1) with aucuba-mosaic virus.

Since the digestion of substances by enzymes is a time reaction, it would appear that the loss of infectivity of virus is not due to the proteolytic activity of trypsin, inasmuch as it took place immediately upon the addition of the trypsin. The fact that the infectivity of the virus-trypsin solution increased on standing, on dilution, and on heating is further evidence that the loss of infectivity is not due to the proteolytic activity of the trypsin. The loss of virus infectivity on addition of trypsinogen is not due to proteolysis, since trypsinogen possesses no proteolytic activity.

The tryptic activity of the solution of trypsin was determined by means of the formol titration, both before and after addition to the virus, and no change was found. Tryptic activity decreased in the trypsin-virus solution after heating and was completely lost after standing for 7 weeks. No tryptic activity was found in any of the trypsinogen solutions at any time.

Since trypsinogen caused a loss of infectivity of the virus, it became of interest to determine whether other proteins would have the same effect. Table 2 gives the results of experiments with 5 different proteins. Here again the protein-treated virus was tested on one side of a leaf against an untreated control on the other side. The untreated virus preparation used in these tests gave an average of about 75 lesions per half-leaf. The results show that egg albumen, pepsin, and gelatin had only a slight effect on the infectivity of the virus at pH 7, whereas globin and commercial

TABLE 2.—*Effect of different proteins on the infectivity of tobacco-mosaic virus*

	pH of solution	Tested on	Immediately	After 3 days at 37° C.	After 7 days at 37° C.	After 14 days at 37° C.	After 25 days at 37° C.
Virus + egg albumin (3 mg. per cc.)	7.0	Phaseolus vulgaris	90.5[a]				
Virus + globin (16 mg. per cc.)	5.8	Nicotiana glutinosa	16.1				
		Phaseolus vulgaris	0.8				
Virus + Parke-Davis pepsin (2 mg. per cc.)	7.0	Nicotiana glutinosa	108.0	84.7	75.1	126.0	
Virus + gelatin (2 mg. per cc.)	7.0	Nicotiana glutinosa	89.0	93.9	69.0	117.0	
		Phaseolus vulgaris	83.5	80.3	90.0	95.0	
Virus + Fairchild trypsin (2 mg. per cc.)	7.0	Nicotiana glutinosa	30.9	66.0	41.4		104.5
		Phaseolus vulgaris	0.0	3.4	7.6		7.5[b]

[a] The numbers represent the average number of lesions on halves of 12 or more leaves of *P. vulgaris* var. Early Golden Cluster and of 5 or more leaves of *N. glutinosa*, expressed as a percentage of the number of lesions obtained with the appropriate control.

[b] This solution was found to possess no tryptic activity.

trypsin caused a great loss in infectivity. Globin, like trypsin and trypsinogen, is peculiar in that it is one of the few proteins that have isoelectric points above pH 7. It may be significant that these three proteins cause a marked decrease in virus infectivity, whereas the proteins that have isoelectric points considerably below pH 7 have no such effect.

In this experiment it may be noted that the commercial trypsin-virus solution possessed no tryptic activity at the end of 25 days at 37° C., whereas the infectivity of the virus as tested on *Nicotiana glutinosa* had been regained until it was comparable to that of the untreated control solu-

TABLE 3.—*Recovery of virus infectivity in a trypsin-virus solution by means of pepsin*

	pH 3		pH 7	
	N. glutinosa	P. vulgaris	N. glutinosa	P. vulgaris
Virus	65.6[a]	3.1 ←	74.2	87.2
Virus + trypsin (0.1 mg. trypsin N per cc.)	10.1	0.0 ←	11.6	0.0
Virus + trypsin + pepsin (0.01 mg. pepsin N per cc.)	5.0	0.0		
Virus + trypsin + pepsin (After 2 days at 37° C.) (No tryptic activity)	46.5		57.5	13.9

[a] The numbers represent the average number of lesions on 12 or more leaves of *P. vulgaris* var. Early Golden Cluster or 10 or more leaves of *N. glutinosa*.

tion. The disappearance of the trypsin and the reactivation of the virus is probably due to the slow denaturation of trypsin which occurs at pH 7 and the digestion of the denatured trypsin by the remaining active trypsin. This process may be assumed to continue until almost all the trypsin has been digested, and as the trypsin is thus removed, the virus is able to manifest its infectivity.

Recovery of Infectivity by Peptic Digestion. Since from the experiments reported above, it appears that the gradual removal of trypsin causes a recovery of virus infectivity, it was decided to determine whether the infectivity could be regained by the rapid digestion of trypsin with pepsin. Table 3 gives the results of the treatment of a trypsin-virus solution with pepsin. In this experiment a purified virus solution was tested at pH 3 and 7, giving the results shown in the first row of figures. To the solution at pH 7 was then added enough of a solution of crystalline trypsin to make 0.1 mg. trypsin nitrogen per cc. This mixture was tested on half-leaves of *Phaseolus vulgaris* and *Nicotiana glutinosa* against the proper controls at pH 3 and 7. It may be seen from table 3 that the preparation produced no lesions on *P. vulgaris*, yet gave an average of 11.6 lesions on *N. glutinosa*. The significance of this differentiation, which has been encountered throughout the work, will be considered in detail in a subsequent section. Enough crystalline pepsin to make 0.01 mg. pepsin nitrogen per cc. was added to the virus solution that had been brought to pH 3. The solution was tested for infectivity on *N. glutinosa* and *P. vulgaris* at pH 3 immediately after the addition of the pepsin. It was then incubated for 2 days at 37° C. and tested at pH 3 and 7 for infectivity and also for tryptic activity. No tryptic activity was found, but the infectivity of the virus had been re-

gained to such an extent that the solution now produced, at pH 7, an average of 13.9 lesions on *P. vulgaris* and 57.5 lesions on *N. glutinosa* as compared to an average of 0.0 and 11.6 lesions, respectively, before the treatment with pepsin. This gain in the infectivity of the virus in the trypsin and pepsin treated solution was such that the solution produced about 16 per cent as many lesions on *P. vulgaris* and 77 per cent as many lesions on *N. glutinosa* as did the original untreated virus solution.

There remained the possibility, however, that the pepsin or the split products of trypsin might have a stimulating effect on the virus, which would account for the increase in infectivity as measured on the 2 test plants. In order to eliminate this possibility pepsin was allowed to act upon trypsin for 66 hours at pH 3 in the relative concentrations mentioned above. Then 1 cc. of this pepsin-trypsin digest was added to 4 cc. of the virus solution and this mixture was tested immediately on leaf-halves against a control, using *Phaseolus vulgaris* and *Nicotiana glutinosa*. Instead of causing an increase in infectivity, this digest caused a slight reduction in the average number of lesions on *N. glutinosa* and a somewhat greater reduction in the number of lesions on *P. vulgaris*. It appears, therefore, that the gain in virus infectivity, which was measured on these plants and was caused by pepsin, represents the minimum rather than the maximum gain in infectivity. However, *N. glutinosa* was least affected by the digest, so the measurements on this plant can be regarded as being more nearly indicative of the real gain in infectivity. Since the virus in the trypsin-pepsin digest gave an average of about 77 per cent as many lesions as did the virus in the untreated control solution, it is obvious that much of the virus infectivity was regained by digestion of the trypsin with pepsin.

The Effect of Different Hydrogen-Ion Concentrations. If the loss of virus infectivity on addition of trypsin is due to proteolysis, it should take place only at hydrogen-ion concentrations at which trypsin is active. Trypsin has its maximum proteolytic activity betwen pH 7.5 and 9.5 and is inactive below about pH 6. Therefore, the effect of trypsin on the infectivity of virus was determined at pH 2.1, 2.7, 4.0 and 5.0, hydrogen-ion concentrations at which trypsin is inactive and also at pH 6.7, 8.0, 10.2 and 10.8, hydrogen-ion concentrations at which trypsin is active. In this experiment a sample of virus, a solution of 0.1 M phosphate and a solution of 0.1 M phosphate containing 0.5 mg. of trypsin nitrogen per cc. were prepared at each of the above hydrogen-ion concentrations. Each virus sample was divided into two equal portions and at each hydrogen-ion concentration an equal volume of 0.1 M phosphate was added to one portion and an equal volume of 0.1 M phosphate containing 0.5 mg. of trypsin nitrogen per cc. was added to the other portion. The hydrogen-ion concentration of each mixture was the same, therefore, as that of the virus sample used in its preparation. The virus preparations at the 8 different hydrogen-

ion concentrations were tested by the half-leaf method against the 8 virus preparations containing 0.25 mg. of trypsin nitrogen per cc. The results (Table 4) show that the hydrogen-ion concentration of the virus preparation has little or no effect on the loss of infectivity, since it took place at all 8

TABLE 4.—*Effect of hydrogen-ion concentration on the loss of virus infectivity upon addition of trypsin*

Tested at pH ⟶		2.1	2.7	4.0	5.0	6.7	8.0	10.2	10.8
P. vulgaris	Virus	7.6[a]	3.3	10.2	33.4	39.6	48.2	57.8	35.3
	Virus plus trypsin (0.25 mg. N per cc.)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N. glutinosa	Virus	19.4	40.1	48.2	47.4	61.6	41.1	9.2	6.0
	Virus plus trypsin (0.25 mg. N per cc.)	1.2	3.3	3.8	3.2	2.0	1.8	1.0	0.0

[a] The numbers represent the average number of lesions on 12 half-leaves of *P. vulgaris* var. Early Golden Cluster or 10 half-leaves of *N. glutinosa*.

hydrogen-ion concentrations. This suggests that the loss of infectivity is not due to the proteolytic activity of trypsin, since it is inactive proteolytically at pH 2.1, 2.7, 4.0 and 5.0.

Studies on Other Effects of Trypsin

If the loss in infectivity of virus on addition of trypsin is not due to proteolysis, it must be explained on some other basis. It seems necessary to assume that trypsin affects either the virus, or the plant, or both. Experiments were therefore made to determine whether trypsin has a direct action on the test plant. Other experiments were made to determine the effect of trypsin on the infectivity of several different viruses, and on the infectivity of tobacco mosaic virus when used to inoculate different species of plants.

The Effect of Different Amounts of Trypsin on the Susceptibility of Plants to the Virus of Tobacco Mosaic. If trypsin acts directly on the plant cells it might be possible to study its action by varying the amount that is added to the virus. Table 5 shows the results of experiments in which solutions of crystalline trypsin were added to untreated juice from mosaic-diseased tobacco plants at pH 5.6 and to a purified virus solution at pH 6.8, and tested immediately with controls on *Phaseolus vulgaris* and *Nicotiana glutinosa*. It may be seen that, while 0.005 or more milligrams

TABLE 5.—*Effect of varying amounts of trypsin on the infectivity of tobacco mosaic virus*

Milligrams of trypsin N per cc. ⟶		0	0.005	0.05	0.25	0.5
Untreated juice from mosaic tobacco plants. Diluted 1 to 6.	P. vulgaris	230.0[a]	17.4	1.0	0.0	0.0
(pH 5.6)	N. glutinosa	303.0	168.0	78.4	21.6	11.8
Purified virus	P. vulgaris	121.0	3.4	0.8	0.0	0.0
(pH 6.8)	N. glutinosa	194.0	88.5	2.0	2.2	0.0

[a] Numbers represent the average number of lesions on 16 leaves of *P. vulgaris* var. Early Golden Cluster or 10 leaves of *N. glutinosa.*

of trypsin nitrogen per cc. has a decided effect when either solution is used, it is more marked in the case of the purified virus solution. Additional tests have demonstrated that even 0.0005 mg. of trypsin nitrogen is very effective when added to a purified virus solution. Lojkin and Vinson (5) reported that they were unable to obtain inactivation of the virus by means of trypsin when they used untreated juice from mosaic-diseased tobacco plants. This difference in results may be due to the use of different enzyme preparations.

TABLE 6.—*Effect of varying amounts of trypsin and virus on infectivity in Phaseolus vulgaris and Nicotiana glutinosa*

Final dilution of virus ⟶		1–2	1–20	1–200
Virus plus 0.25 mg. trypsin N per cc.	P. vulgaris	0.0[a]	0.0	0.0
	N. glutinosa	10.8	1.9	0.1
Virus plus 0.125 mg. trypsin N per cc.	P. vulgaris	0.0	0.0	0.0
	N. glutinosa	14.3	5.5	0.3
Virus plus 0.025 mg. trypsin N per cc.	P. vulgaris	0.5	0.5	0.0
	N. glutinosa	46.6	29.6	1.4
Virus plus 0.0025 mg. trypsin N per cc.	P. vulgaris	15.8	6.9	0.5
	N. glutinosa	45.8	55.5	6.2
Virus plus 0.00125 mg trypsin N per cc.	P. vulgaris		17.8	14.7
	N. glutinosa		46.8	8.7
Virus plus no trypsin	P. vulgaris	55.0	49.0	14.9
	N. glutinosa	65.0	65.0	8.2

[a] The numbers represent the average number of lesions on 12 or more half-leaves of *P. vulgaris* var. Early Golden Cluster or 5 or more half-leaves of *N. glutinosa.*

Studies were then made with varying amounts of virus as well as of trypsin. To purified solutions of virus in 0.1 M phosphate buffer at pH 6.8 were added equal volumes of 0.01 M HCl containing from zero to 0.5 mg. of trypsin nitrogen per cc. The virus solution was sufficiently well buffered that the addition of the acid caused no appreciable change in the pH. The final dilution of the virus solution was 1 part in 2, 1 in 20, and 1 in 200. The amount of trypsin was varied from 0.25 to 0.0025 mg. trypsin nitrogen per cc. It may be seen from table 6 that trypsin caused a decrease in the number of lesions at all concentrations used and that this decrease was greater with *Phaseolus vulgaris* than it was with *Nicotiana glutinosa*. The two highest concentrations of trypsin caused the virus solution to lose completely its infectivity for *P. vulgaris*, although these same solutions produced lesions on *N. glutinosa*. This phenomenon, as was previously mentioned, has been encountered throughout the work. The fact that it is possible to prepare a virus-trypsin solution that will produce local necrotic lesions on one plant species and yet fail to produce lesions on a second equally or more susceptible species is a definite indication that trypsin is affecting the second test plant. It has been demonstrated repeatedly that a virus solution that has been diluted with distilled water or phosphate at pH 7 so that it will give an average of approximately 10 lesions per leaf on *N. glutinosa* will also give an average of at least 10 and usually more lesions per leaf on *P. vulgaris*. The results of the tests of the control virus solutions as given in tables 1, 6, and 7 may be cited as examples. It may be seen from these tables that, at this concentration of virus, plants of *P. vulgaris* respond to inoculation with as many lesions as do *N. glutinosa* plants. Therefore, the production of lesions on *N. glutinosa* on inoculation with the virus-trypsin solution and the failure of the same solution to produce lesions on *P. vulgaris* is definite evidence that trypsin is affecting the plants of *P. vulgaris*.

Similar results were obtained when a more concentrated virus solution, which permitted greater dilution, was used, as may be seen from table 7. In this experiment the trypsin nitrogen concentration was varied between 0.0005 mg. and 0.1 mg. per cc. and the dilutions of virus were carried out to 1 part in 200,000. In many instances at higher trypsin concentrations or lower virus concentrations, solutions were obtained that gave lesions on *Nicotiana glutinosa* and yet failed to produce lesions on *Phaseolus vulgaris*. In order to gain additional information concerning the difference in response to trypsin of these two plant species an attempt to correlate the data given in table 7 was made. The number of lesions obtained with the control or untreated virus solution was regarded as 100 per cent at each dilution and the percentage drop in the number of lesions from this number at each dilution was calculated. This percentage drop in the number of

TABLE 7.—*The effect of a constant amount of trypsin on varying amounts of virus*

Dilution of virus ⟶	Test plant ↓	1–2	1–20	1–200	1–2000	1–20,000	1–200,000
Virus	P. vulgaris	295.8[a]	135.9	65.6	11.4	1.16	0.3
	N. glutinosa	214.0	97.0	32.2	2.05	0.2	0.0
Virus plus 0.0005 mg. trypsin N per cc.	P. vulgaris	95.5	30.5	6.5	0.28	0.0	0.0
Virus plus 0.001 mg. trypsin N per cc.	P. vulgaris	54.0	18.4	2.0	0.0	0.0	0.0
	N. glutinosa	147.0	60.2	28.7	1.6	0.2	0.0
Virus plus 0.002 mg. trypsin N per cc.	N. glutinosa	141.5	67.3	10.6	1.35	0.06	0.0
Virus plus 0.005 mg. trypsin N per cc.	P. vulgaris	15.4	3.6	0.4	0.0	0.0	0.0
	N. glutinosa	176.0	42.3	9.2	1.3	0.1	0.0
Virus plus 0.05 mg. trypsin N per cc.	P. vulgaris	0.5	0.0	0.0	0.0		
	N. glutinosa	47.3	6.6	2.8	0.4	0.09	0.0
Virus plus 0.1 mg. trypsin N per cc.	P. vulgaris	0.5	0.0	0.0			
	N. glutinosa	16.5	2.7	1.3	0.1		

[a] The numbers represent the average number of lesions on 12 or more half-leaves of *P. vulgaris* var. Early Golden Cluster or *N. glutinosa.*

lesions was plotted (see Figs. 1 and 2) against the dilution of the virus on a double logarithmic scale, for each trypsin concentration and for each test plant. It may be seen from figure 1 that with this virus solution, concentrations of from 0.001 mg. to 0.1 mg. trypsin nitrogen per cc., gave lines approximately paralleling that of the control when tested on *N. glutinosa.* This indicates that these trypsin concentrations produce constant percentage reductions in the number of lesions on *N. glutinosa.* The results as given in figure 2 show that trypsin concentrations of 0.0005 mg. trypsin nitrogen per cc. or more gave progressively increasing percentage drops in the number of lesions when tested on *P. vulgaris.* The difference in the response of the two test plants to trypsin may be seen by a comparison of the curves for trypsin nitrogen concentrations of 0.001 and 0.005 mg. in figures 1 and 2. In these cases a constant reduction at all dilutions was obtained on *N. glutinosa,* whereas the same solutions gave progressively increasing reductions on dilution of the virus when tested on *P. vulgaris.*

The fact that it is possible to obtain a fairly constant reduction in the number of lesions on *Nicotiana glutinosa* at several dilutions of virus with a given constant amount of trypsin seems to indicate that trypsin is acting

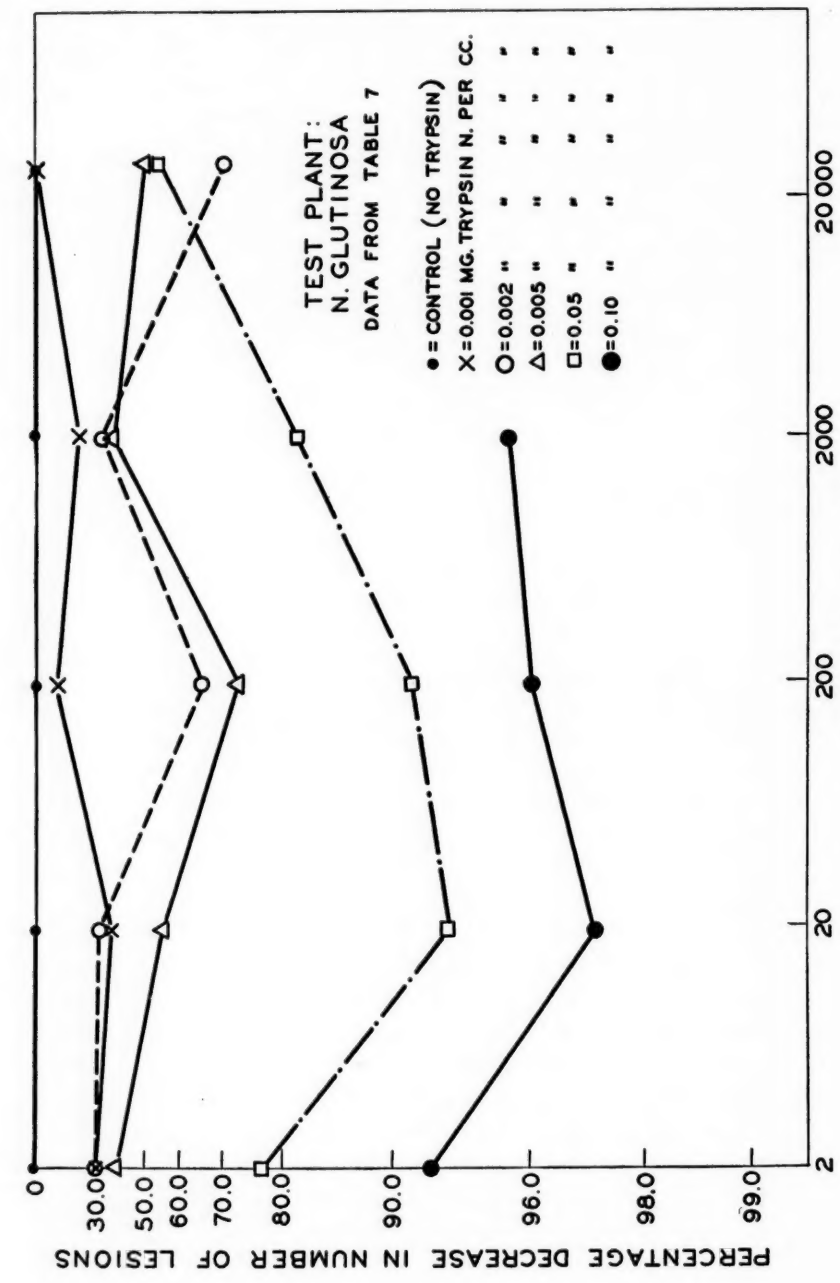

FIG. 1. Graph showing the percentage decrease in the average number of lesions on 12 or more leaves of *Nicotiana glutinosa* L. upon inoculation with tobacco-mosaic virus at several dilutions in the presence of 5 different concentrations of trypsin. In general the lines are parallel. This shows that at these concentrations, trypsin causes a fairly constant percentage reduction in the number of lesions on *N. glutinosa* upon dilution of the virus.

on the plant. However, the fact that at certain higher concentrations of trypsin it is possible to demonstrate that a given amount of trypsin causes a progressively increasing percentage drop in the number of lesions, on dilution of the virus, indicates that here there may be some action of trypsin on the virus, since as the virus concentration is reduced, the inhibitory effect of the trypsin becomes greater. However, if the progressively in-

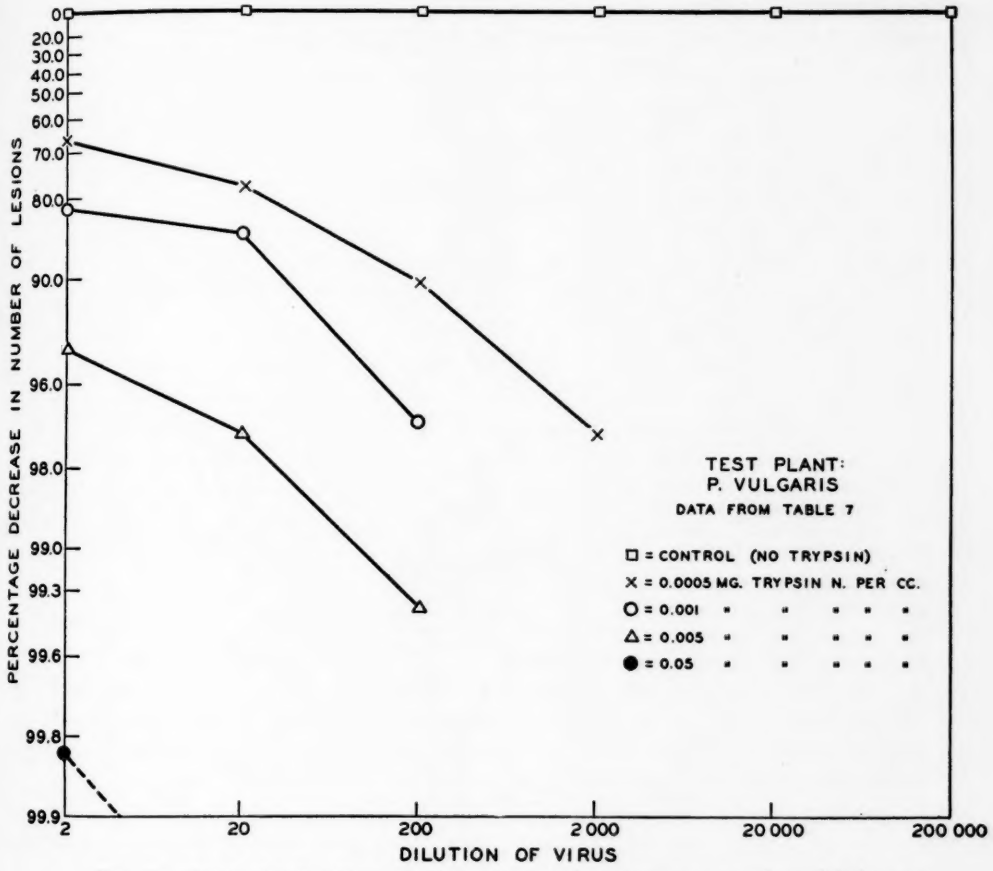

FIG. 2. Graph showing the percentage decrease in the average number of lesions on 12 or more leaves of *Phaseolus vulgaris* L. upon inoculation with tobacco-mosaic virus at several dilutions in the presence of 4 different concentrations of trypsin. The drop in the 4 lines depicting 4 trypsin concentrations shows that at these concentrations, trypsin causes progressively increasing percentage reductions in the number of lesions on *P. vulgaris* upon dilution of the virus.

creasing drop in the number of lesions obtained when a virus solution containing 0.001 mg. trypsin nitrogen is tested on *Phaseolus vulgaris* is to be regarded as evidence that trypsin is acting on the virus, it is difficult to

percentage reduction in the number of lesions on *N. glutinosa* upon dilution of the virus.

understand why this same drop, indicative of effect on virus, does not appear when this same solution is tested on *N. glutinosa.* Here the evidence seems to indicate that trypsin is affecting only the test plant, since the percentage reduction in the number of lesions is the same at several dilutions of the virus. Some secondary and as yet undemonstrated factor may be causing this phenomenon. There seems to be no doubt, however, that trypsin does act directly on *N. glutinosa* and *P. vulgaris* to reduce their susceptibility to the virus of tobacco mosaic, and that the intensity of the action of trypsin is not the same for these two plant species.

Effect of Trypsin on the Susceptibility of Different Species of Plants to the Virus of Tobacco Mosaic. Since trypsin was found to affect the susceptibility of plants to the virus of tobacco mosaic, the action being more pronounced with *Phaseolus vulgaris* than with *Nicotiana glutinosa,* it seemed possible that the action might vary to an even greater extent with other plants. In fact, it did not appear impossible that some plant might be entirely resistant to trypsin and hence be as susceptible to virus plus trypsin as to virus alone. In order to test this possibility the right halves of leaves of one or more plants of *Nicotiana acuminata* Hook., *N. langsdorffii* Schrank, *N. rustica* L. var. Winnebago, *N. tabacum* var. *purpurea* × *glutinosa, Datura stramonium* L., *Solanum melongena* L. var. Black Beauty, *S. pseudo-capsicum* L., *Physalis angulata* L., and *Capsicum frutescens* L. were rubbed with a sample of purified virus. The left halves of the same leaves were rubbed with a similar sample of virus plus 0.5 mg. of trypsin nitrogen per cc. The test was repeated, using a sample of untreated infectious juice from mosaic-diseased Turkish tobacco plants instead of the purified virus. This sample was tested against untreated infectious juice plus 0.5 mg. trypsin nitrogen per cc. The results, some of which may be judged from figure 3, were the same in all instances. The virus caused many necrotic lesions on the right sides of the leaves where inoculation was with virus alone, and none or at most 1 or 2 lesions on the left sides where inoculation was carried out in the presence of trypsin. The plants listed above represent a rather wide range of species, yet all of the plants were found to be affected by trypsin. Therefore, although the intensity of the inhibitory action of trypsin on the susceptibility of plants to the virus of tobacco-mosaic may vary somewhat with the species of test plant used, the inhibitory action appears to be more or less general, since all plants used were affected by trypsin.

Effect of Adding Trypsin after Inoculation with Virus. It seemed of interest to determine whether a solution of trypsin, rubbed or sprayed on the leaves of inoculated plants at different intervals following inoculation, would affect the infectivity of the virus. The entire leaves of 34 sets of 1 or more plants each of *Nicotiana glutinosa* and *Phaseolus vulgaris* were in-

oculated by rubbing with a purified preparation of tobacco-mosaic virus. The inoculated leaves were then washed off in running tap water, with the exception of the leaves of two sets of plants which were not washed. Instead of using the half-leaf method, 1 leaf of a plant of *P. vulgaris* was treated and the other leaf of the same plant served as a control. In the case of *N. glutinosa* 2 or 3 leaves were treated and 2 or 3 similar leaves of

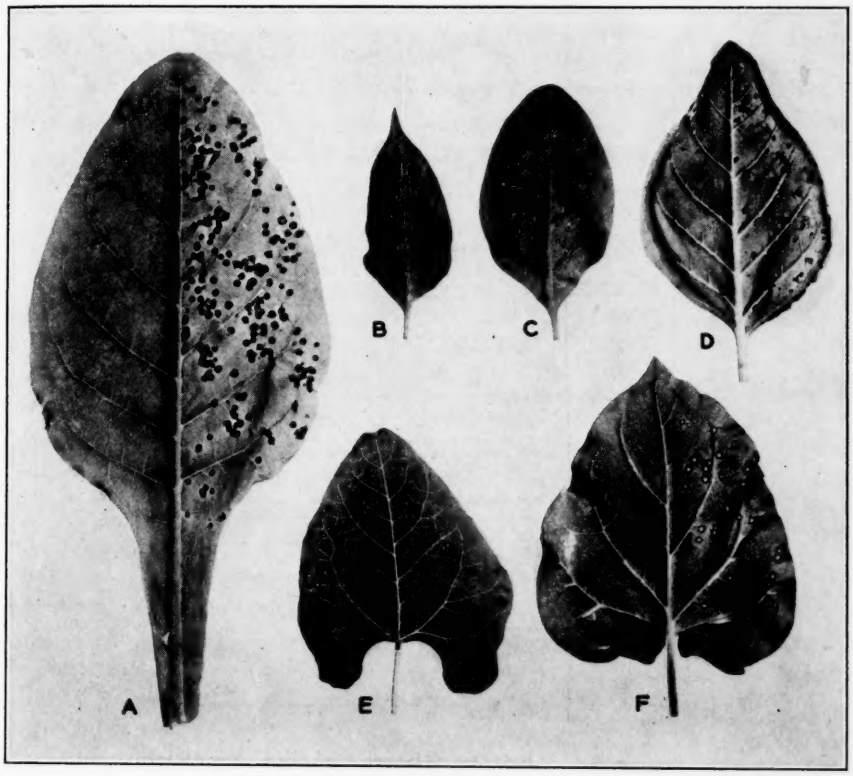

FIG. 3. Leaves of (A) *Nicotiana langsdorffii* Schrank, (B) *Capsicum frutescens* L., (C) *N. tabacum* L. var. *purpurea* × *glutinosa*, (D) *N. acuminata* Hook., (E) *Phaseolus vulgaris* L. var. Early Golden Cluster, and (F) *N. glutinosa* L. as they appeared 5 days after inoculation. The right halves of the leaves were inoculated with tobacco-mosaic virus and the left halves with another portion of the same tobacco-mosaic virus preparation immediately after the addition of 0.5 mg. of trypsin nitrogen per cc. The leaves are not susceptible to virus in the presence of trypsin as is shown by the absence of lesions in the portions of the leaves inoculated with virus plus trypsin.

the same plant were used as controls. Therefore, in one set, one leaf of each plant of *P. vulgaris* was rubbed immediately with a trypsin solution containing 0.5 mg. trypsin nitrogen per cc., and the other leaf of the same plant was rubbed with distilled water. In the case of *N. glutinosa* 2 or 3

TABLE 8.—*Effect of rubbing or spraying trypsin at various time intervals after inoculation of virus*

Time interval in min., between inoculation of virus and administration of trypsin	Trypsin added by rubbing						Trypsin added by spraying					
	P. vulgaris			N. glutinosa			P. vulgaris			N. glutinosa		
	Control	Trypsin treated	Trypsin ÷ Control	Control	Trypsin treated	Trypsin ÷ Control	Control	Trypsin treated	Trypsin ÷ Control	Control	Trypsin treated	Trypsin ÷ Control
Immediately	31[a]	2[a]	6[b]	187[a]	37[a]	20[b]						
5	47	7	15	50	29	58	41[a]	2[a]	5[b]	38[a]	14[a]	37[b]
10	33	3	9	61	20	33	64	4	6	150	79	53
20	60	7	12	42	22	52	82	5	6	235	108	46
30	94	40	43	23	12	52	42	17	40	41	30	73
40	204	144	70	84	45	54	217	112	52	119	97	81
60	129	76	59	83	36	43	129	97	75	133	59	44
75	121	90	74	92	48	52	150	115	76	237	175	74
90	124	101	81	65	35	54	100	58	58	187	125	67
110	138	137	99	108	50	46	153	129	84	202	130	64
120	153	137	90	225	200	89	137	118	86	300	225	75
150	150	124	83	50	35	70	170	125	73	105	89	85
180	209	192	92	78	48	61	163	140	86	226	197	87
240	163	120	74	40	26	65	130	114	88	191	164	86
300	183	171	94	103	78	75	129	115	89	225	200	89
420	166	158	95	113	106	94	109	97	89	325	300	92
1 day	200	181	90	260	256	98	229	210	92	234	222	95

[a] Numbers represent the average number of lesions on 5 or more leaves of *P. vulgaris* var. Early Golden Cluster or 2 or more leaves of *N. glutinosa.*

[b] Numbers represent the average number of lesions per leaf on the trypsin-treated leaves divided by the average number of lesions per leaf on the corresponding control leaves multiplied by 100.

leaves were treated with the trypsin solution and 2 or 3 similar leaves of the same plant were rubbed with distilled water. This was repeated with the other set except that trypsin and water were administered by spraying from an atomizer instead of by rubbing. The leaves were then washed off in running tap water. The rest of the inoculated *P. vulgaris* and *N. glutinosa* plants, which had been washed in running tap water, were divided into 32 similar sets. At various intervals of from 5 minutes to 1 day after inoculation with virus, sets of plants were similarly treated by rubbing and by spraying with a trypsin solution and with distilled water. At the end of 5 days the average number of lesions on the trypsin-treated leaves was compared with the average number of lesions on the control or water-treated leaves. The results are given in table 8. The numbers in the columns designated by "Trypsin ÷ Control" represent the average number of lesions per leaf on the trypsin-treated leaves divided by the average number of lesions per leaf on the control leaves multiplied by 100, and hence may be regarded as a measure of the inhibitory effect of trypsin. It may be seen that trypsin was most effective on *P. vulgaris* during the first 30 minutes, at which time an abrupt decrease in inhibitory effect occurred, followed by a further gradual decrease until there was practically no effect. The effect on *N. glutinosa* was not so pronounced during the first 30 minutes and the subsequent decrease in the inhibitory effect was fairly gradual.

It was then decided to determine if spraying the trypsin solution on uninoculated leaves would have an effect on the subsequent inoculation of the leaves with virus. Accordingly, in this experiment, the leaves of 18 *Phaseolus vulgaris* plants and 8 *Nicotiana glutinosa* plants were sprayed with water, and the leaves of a like number of plants were sprayed with a solution prepared by dissolving 1 gm. of commercial trypsin in 50 cc. of water and adjusting to pH 6. The plants were watered from below so as not to wet the leaves until after the inoculation of the plants with virus. Six trypsin-sprayed *P. vulgaris* and 2 trypsin-sprayed *N. glutinosa* plants, and a like number of the water-sprayed plants, were then inoculated with a virus solution after 2 days, and similar sets were inoculated at the end of 4 and 6 days. The results as given in table 9 show that the sprayed trypsin caused a large decrease in the number of lesions. No lesions whatsoever appeared on the trypsin-sprayed leaves of *P. vulgaris*, even when there was a 6-day interval between spraying of trypsin and inoculation of virus. There was some tendency for the number of lesions on the trypsin-sprayed *N. glutinosa* leaves to increase as the time period was lengthened from 2 to 6 days.

Since no lesions appeared on the trypsin-sprayed leaves of *Phaseolus vulgaris*, it seemed desirable to determine if infection of Turkish tobacco plants with tobacco-mosaic virus could be prevented by a preliminary

TABLE 9.—*Effect of trypsin when sprayed on leaves before inoculation of virus*

Inoculated after ——→		2 days	4 days	6 days
Plants sprayed with water	P. vulgaris	45.0[a]	46.2	38.7
	N. glutinosa	112.5	63.0	43.0
Plants sprayed with a trypsin solution	P. vulgaris	0.0	0.0	0.0
	N. glutinosa	5.3	8.5	15.0

[a] Numbers represent the average number of lesions on 12 leaves of *P. vulgaris* var. Early Golden Cluster or 10 leaves of *N. glutinosa*.

spraying of the tobacco plants with a solution of trypsin. In the previous experiment the trypsin-sprayed leaves were not watered by sprinkling from above until after the inoculation with virus, so in this experiment it was decided to determine the effect of watering. Accordingly, 20 flats of small Turkish tobacco plants containing 48 plants in each flat were divided into 4 groups of 5 flats each. One group was left untreated and was watered by sprinkling from above. The second group was left untreated and was watered from below, so as not to wet the leaves. The third group was sprayed thoroughly with a trypsin solution similar to that used in the preceding experiment and then watered by sprinkling from above. The last group was sprayed with trypsin, but was watered from below, so as not

TABLE 10.—*Infection of trypsin-treated Turkish tobacco plants with tobacco-mosaic virus*

Inoculation after →	1 day	2 days	3 days	4 days	5 days
(Untreated) watered from below	100.0[a]	96.0	83.3	87.3	93.6
(Untreated) watered from above	98.0	91.6	81.2	83.0	98.0
Sprayed with trypsin and watered from below	0.0[b]	4.2[b]	4.2[c]	6.2	14.6
Sprayed with trypsin and watered from above	12.5[b]	29.1[c]	62.5	87.5	81.2

[a] Numbers represent the per cent of infected plants out of 48 inoculated.

[b] After 10 days the diseased plants were removed and the healthy plants were reinoculated and the percentage infection was 83.2, 82.5 and 90.5, respectively.

[c] After 10 days the diseased plants were removed and the healthy plants left uninoculated. All remained healthy.

to wet the leaves. One flat from each of the 4 groups was inoculated by rubbing 1 leaf of each plant once with infectious juice diluted 1–1000 with water 1 day after the spraying treatment. This was repeated after 2, 3, 4 and 5 days, using 1 flat from each of the 4 groups each day. Table 10 gives the percentage infection in each flat caused by this treatment. A high percentage of infection occurred in every flat that had not been treated with trypsin, whereas the infection was low in the flats that were inoculated 1 day after being sprayed with trypsin. The percentage infection rose as the interval between spraying and inoculation was increased, especially in the case of the flats watered by sprinkling from above. In the latter case the percentage infection was considerably higher at every time interval, and after 4 or 5 days it was comparable to that of the non-trypsin-treated controls. After 10 days a number of the healthy plants were reinoculated with virus and a high percentage of infection resulted, indicating that the trypsin treatment failed to produce any permanent protection. The results indicate that trypsin is lost from the leaves very slowly if the leaves are not washed, but that it is lost fairly rapidly if the leaves are washed. Furthermore, the trypsin must be present on the leaves at the time of inoculation of the virus or shortly thereafter in order to manifest its inhibitory action.

In order to obtain more definite information, it was decided to rub a trypsin solution over the leaves of *Nicotiana glutinosa* and *Phaseolus vulgaris* plants and test for the presence of trypsin on these leaves after time intervals, during which one set had been washed and watered as usual and the other set left unwashed and unwatered, except from below, so as not to wet the leaves. The tests for the presence of trypsin were made by inoculating sets of the plants with virus after the various time intervals and also by testing either the wash water from the leaves or an extract of the ground-up leaves for tryptic activity and for inhibitory action when added to a virus solution.

The leaves of 36 *Phaseolus vulgaris* plants and 6 *Nicotiana glutinosa* plants were rubbed 5 times each with a piece of gauze saturated with water. These plants, which were watered by sprinkling from above, served as controls and are designated as "Lot C." The leaves of 108 *P. vulgaris* plants and 18 *N. glutinosa* plants were rubbed 5 times each with a piece of gauze saturated with a solution prepared by dissolving 1 gm. of commercial trypsin in 50 cc. of distilled water and adjusting to pH 6. The leaves of half of these plants were then washed thoroughly in running water and watered from above. These washed and watered plants are designated as "Lot A." The leaves of the other half of the plants were not washed, the plants being watered from below. The plants from which the trypsin solution was not washed are designated as "Lot B." Four hours after the above de-

scribed treatment with water or a trypsin solution, 8 *P. vulgaris* plants and 2 *N. glutinosa* plants from each of lots A, B and C were inoculated with the same virus solution. This inoculation with virus was repeated on additional plants of lots A, B and C, 24 and 48 hours after the first treatment with water or trypsin. The average number of lesions appearing on 1 leaf from each of the 3 lots inoculated on the 3 successive days is given in table 11.

TABLE 11.—*Effect of washing trypsin-treated leaves*

Inoculated with virus ⟶		After 4 hours	After 24 hours	After 48 hours
Lot A, plus trypsin (washed)	P. vulgaris	0.2[a]	0.2	3.4
	N. glutinosa	23.6	42.1	64.4
Lot B, plus trypsin (unwashed)	P. vulgaris	0.0	0.0	0.1
	N. glutinosa	14.1	29.4	35.3
Lot C, control	P. vulgaris	77.0	164.0	142.0
	N. glutinosa	117.5	85.4	81.0

[a] The numbers represent the average number of lesions on 16 leaves of *P. vulgaris* var. Early Golden Cluster or 10 leaves of *N. glutinosa*.

The results show that a preliminary treatment of the leaves with trypsin either 4, 24, or 48 hours before the inoculation of the leaves with virus caused a marked decrease in the number of lesions produced by the virus. The shorter the time elapsing between the trypsin treatment and the inoculation, the greater was the effect produced by the trypsin. More lesions appeared after the 48-hour time period than after either the 24- or 4-hour period. The increase in the number of lesions as the period of time was lengthened indicates that the effective trypsin was being removed or destroyed slowly. The fact that more lesions were produced on the washed and watered leaves than on the unwashed leaves indicates that washing does remove some, but not all, of the trypsin from the leaves.

It seemed of interest to determine whether or not the trypsin actually remains on the leaves, and if so, whether or not it is in an active condition, and also to gain some idea of the location of such trypsin. Accordingly the leaves from 18 *Phaseolus vulgaris* plants and 3 *Nicotiana glutinosa* plants from lots A and B were used to prepare aqueous extracts by 3 different extraction procedures 24 hours after the trypsin treatment. Procedure No. 1 consisted of rubbing the surface of the leaves under a little water thoroughly with a small piece of gauze. It was hoped that this would remove all the trypsin which was on the surface of the leaves. Procedure No. 2 consisted of applying vacuum and then pressure to leaves placed first

under water and then out of water. It was hoped that this treatment would remove trypsin from the intercellular spaces and hair cells. The last procedure, No. 3, consisted of grinding up the entire leaf and preparing an aqueous extract from the macerated plant tissue in the expectation of obtaining all the trypsin from the leaves, regardless of its location. The amount of trypsin present in each of these 12 extracts was then determined by testing directly for tryptic activity by the formol titration and also by determining the reduction in the number of lesions produced when 5 cc. of each of the extracts was added to 5 cc. of a sample of virus and inoculated on leaf-halves against a control consisting of the virus diluted with an equal volume of water.

A comparison of the results obtained by the 2 methods of testing was desirable, hence table 12 was arranged to give the results of each test on a

TABLE 12.—*Comparison of chemical and plant tests for trypsin*

Extract from ↓	Extraction method	Lot A		Lot B	
		Formol[a] titration	Lesions on[b] P. vulgaris	Formol[a] titration	Lesions on[b] P. vulgaris
P. vulgaris	1	0.0	1.5	34.8	48.5
N. glutinosa	1	0.0	0.0	69.5	70.0
P. vulgaris	2	8.7	25.0	52.1	63.8
N. glutinosa	2	8.7	46.5	26.1	61.2
P. vulgaris	3	8.7	20.5	100.0	94.4
N. glutinosa	3	26.1	51.0	82.5	96.3

[a] Numbers directly proportional to the number of cc. of 0.02 N NaOH used.
[b] Numbers inversely proportional to the actual number of lesions.

percentage basis, the extract requiring the greatest amount of 0.02 N sodium hydroxide in the formol titration being listed as 100.0 and the remaining titrations being based on this number. The more trypsin present, the greater is the amount of sodium hydroxide needed in the formol titration and the larger the number in the table; in the test on *Phaseolus vulgaris* the more trypsin present, the fewer are the number of lesions, thus, in this case, the numbers are inversely proportional to the number of lesions. It may be seen that, in general, the results obtained by the formol titration method for trypsin are in fairly close agreement with those obtained by testing the solutions for their effect on the infectivity of the virus. The results also indicate that much active trypsin remained on the surface of the leaf when it was not washed, but that washing the leaves or watering the plants by sprinkling removed nearly all of this trypsin. Furthermore, after the trypsin had been removed by simple washing there remained ac-

tive trypsin on or in the leaf that could not be rubbed or washed off, the presence of which could be demonstrated by applying pressure and vacuum to remove it from the leaf or by thoroughly macerating the leaf and then pressing out the liquid. It has been demonstrated, therefore, that trypsin sprayed on a leaf exerts its inhibitory effect for periods as long as one week if the leaf is not washed, but that such trypsin can be removed by thorough washing and does not interfere with a subsequent inoculation of virus. However, the treatment of a leaf with a trypsin solution by rubbing fixes the trypsin in or on the leaf, so that some of the trypsin remains on the leaf and cannot be removed without destroying the leaf. This trypsin interferes with a subsequent inoculation of the leaf with virus.

The Effect of Trypsin on the Susceptibility of Plants to Different Viruses. Since the inhibitory effect of trypsin on the susceptibility of plants to tobacco-mosaic virus has been demonstrated, using many different species of plants as hosts, and since other experiments indicate that this effect is due to the action of trypsin on the host plant, attempts were made to demonstrate this inhibitory effect with different strains of tobacco-mosaic virus and with entirely different viruses. The viruses used in these experiments were severe etch, tobacco ring-spot, ordinary cucumber mosaic, a yellow strain[3] of cucumber-mosaic (10), aucuba-mosaic, and two other widely differing strains of tobacco-mosaic, namely a masked (3) and a yellow (4) strain.[4] All viruses were obtained from infected Turkish tobacco plants, except aucuba-mosaic virus, which was from infected tomato plants. The masked tobacco-mosaic, yellow tobacco-mosaic, and aucuba-mosaic viruses were tested on leaf-halves of *Phaseolus vulgaris* and *Nicotiana glutinosa.* Samples of untreated infectious juice plus an equal volume of 0.01 N HCl were used as controls and tested against similar samples of infectious juice plus an equal volume of 0.01 N HCl containing varying amounts of trypsin. The final trypsin-treated juice samples contained 0.5, 0.1 or 0.01 mg. trypsin nitrogen per cc. Severe etch was tested on Turkish tobacco, ring-spot on cowpea, *Vigna sinensis* Endl. var. Black Eye, and the two strains of cucumber-mosaic virus were tested on both Turkish tobacco and *V. sinensis.* The tests on Turkish tobacco were made by inoculating one flat of 48 plants by rubbing one leaf of each plant once with gauze dipped in infectious juice diluted 1 to 50 with water and a second flat with a similar sample of juice diluted 1 to 50 with water and containing 0.1 mg. trypsin nitrogen per cc. The tests on *V. sinensis* were made by inoculating 15 or more plants by rubbing the leaves with gauze dipped in infectious juice and 3 similar sets of plants with 3 additional samples of the same infectious

[3] Designated by Dr. W. C. Price as strain No. 6.

[4] This virus produces large, sharply defined, yellow primary lesions, and yellow mosaic mottling and distortion on systemically infected leaves of Turkish tobacco plants.

juice containing 0.5, 0.1 and 0.01 mg. trypsin nitrogen per cc., respectively. The average number of lesions per half-leaf or per plant obtained on plants of *P. vulgaris, N. glutinosa* and *V. sinensis* with controls or nontrypsin-treated samples of infectious juice was compared with the average number of lesions obtained with the corresponding trypsin-treated samples of infectious juice. When Turkish tobacco was used as the test plant the percentage of infected plants obtained upon inoculation of untreated juice was

TABLE 13.—*Effect of different amounts of trypsin on the susceptibility of plants to different viruses*

Milligrams trypsin nitrogen per cc. →		0.5		0.1		0.01	
	Test plant	Control	Trypsin	Control	Trypsin	Control	Trypsin
Tobacco mosaic (yellow strain)	P. vulgaris	144[a]	0	187	0.5	206	1.5
	N. glutinosa	157[a]	8	202	30	206	92
Tobacco mosaic (masked strain)	P. vulgaris	88[a]	0	148	0.3	100	1.1
	N. glutinosa	225[a]	12	185	26	153	108
Aucuba mosaic	P. vulgaris	23[a]	0	17	0	7	0.5
	N. glutinosa	95[a]	3	38	10	35	26
Cucumber mosaic (Ordinary)	V. sinensis	8.8[b]	0	8.8	0	8.8	0
	Turkish			85[c]	0		
Cucumber mosaic (yellow strain)	V. sinensis	5.4[b]	0	5.4	0	5.4	0
	Turkish			34[c]	0		
Severe etch	Turkish			77[c]	0		
Tobacco ring-spot	V. sinensis	9.4[b]	0	9.4	0	9.4	0

[a] Numbers represent the average number of lesions on 12 half-leaves of *P. vulgaris* var. Early Golden Cluster, or 10 half-leaves of *N. glutinosa.*

[b] Numbers represent the average number of lesions on 15 or more plants of *V. sinensis.*

[c] Numbers represent the percentage of infected plants out of 48 Turkish tobacco plants.

compared with the percentage of infected plants obtained upon inoculation of the corresponding trypsin-treated preparation. The results (Table 13) show that a marked reduction in susceptibility of plants occurred upon addition of trypsin, regardless of the virus used. The results also indicate that, in general, larger amounts of trypsin cause plants to be less susceptible to the virus. The difference in response of *N. glutinosa* and *P. vulgaris* to the same trypsin-virus sample, which has been mentioned previously, is again evident. For example, a sample of masked strain of tobacco-mosaic virus plus 0.01 mg. trypsin nitrogen per cc., which gave an average of 108 lesions per half-leaf on *N. glutinosa,* gave only 1.1 lesions per half-leaf on *P. vulgaris.* This same virus sample plus 0.5 mg. trypsin nitrogen per cc. gave 12 lesions on *N. glutinosa* and no lesions on *P. vulgaris.* It has been demonstrated, therefore, that plants, susceptible to one or more of two widely differing strains of tobacco-mosaic, aucuba-mosaic, two strains of cucumber-mosaic, tobacco ring-spot, and severe etch, become much less susceptible when virus is inoculated in the presence of trypsin.

The Rate of Diffusion of Trypsin in the Presence of Tobacco-mosaic Virus. Most of the evidence from the experiments described in the foregoing paragraphs has indicated that trypsin affects the test plant used to measure the infectivity of the virus. No definite evidence that trypsin affects the virus has been obtained, although some of the results could be interpreted on this basis. Since it has been demonstrated that trypsin does not inactivate the virus by its hydrolytic action, any direct inactivating action of trypsin would suggest an inactive combination or association complex of virus with trypsin. The isoelectric point of the virus is not known with certainty, although some investigators (13) consider that it is negatively charged in the range above about pH 3. If the virus does possess a negative charge above pH 3 it does not appear impossible that inactivation might be brought about on the formation of a complex by the union of oppositely charged units, since proteins having a high isoelectric point are positively charged in this range. In such a case the complex would be different from a simple adsorption complex, since when activated charcoal in the proper amount is added to a virus solution the apparent infectivity increases, yet it may be demonstrated that most of the virus is adsorbed on the charcoal. It appears that something other than simple adsorption is necessary to account for loss of virus infectivity, yet the reaction cannot be too involved, since the virus infectivity may be regained by the removal of trypsin.

Perhaps the simplest example of a phenomenon that might be regarded as analogous is the reaction between heme and globin. In this case heme is not active when free and gains its physiological activity only when in combination with globin, a protein having an isoelectric point above pH 7.

This combination of heme with globin is readily reversible. If there is a similar combination of virus with trypsin, the properties of trypsin in combination should be different from the properties of free or ordinary trypsin. It would be expected that the rate of diffusion of a virus-trypsin complex would be different from the rate of diffusion of trypsin. It was decided, therefore, to determine the rate of diffusion of trypsin in the presence of tobacco-mosaic virus. Since the size of the virus is not known with certainty and since nothing is known concerning the probable ratio of virus to trypsin in such a complex, it is impossible to be certain that any given virus preparation contains enough virus to combine with all of the trypsin molecules in a solution containing but a few milligrams of trypsin nitrogen per cubic centimeter. It is necessary, of course, that most of the trypsin be in combination if a difference in the rate of diffusion is to be found. Although it would be impossible to be certain that all or most of the trypsin molecules have gone into combination with virus, the most favorable experimental conditions would consist of a very dilute trypsin solution plus a very concentrated virus solution. However, for the diffusion experiments, which were performed using a Northrop-Anson (7) diffusion cell, it was necessary to have sufficient trypsin present so that the amount diffusing represented a measurable amount and but a very small percentage of the total trypsin present in the cell. Nevertheless, it was possible to drop the trypsin concentration to about 1 mg. of trypsin nitrogen per cubic centimeter. A concentrated purified virus preparation capable of producing lesions on *Phaseolus vulgaris* when diluted 10^{-8} was used.

The rates of diffusion were determined by the method described by Northrop and Anson (7) and used by Scherp (12) to determine the diffusion coefficient of crystalline trypsin. A solution of crystalline trypsin in 0.5 saturated magnesium sulphate plus 0.1 M sodium acetate at pH 4 containing about 2 mg. of trypsin nitrogen per cc. was prepared. To one volume of this solution was added an equal volume of the concentrated purified virus preparation in 0.1 M phosphate at pH 4. To another volume of the trypsin solution was added an equal volume of 0.1 M phosphate at pH 4. Outside the cell at the start of the experiments was a solution at pH 4, containing 0.25 saturated magnesium sulphate, 0.05 M sodium acetate and 0.05 M phosphate. The cell was first filled with the solution containing trypsin, but no virus, and the amount of trypsin diffusing across the glass membrane in successive 18-hour intervals was determined.[5] The cell was then filled with the solution containing trypsin plus virus and the amount of trypsin diffusing across the glass membrane in successive 18-hour intervals was again determined. The amount of trypsin diffusing across

[5] The trypsin determinations were made by Dr. M. Kunitz and Miss Margaret MacDonald.

the membrane from the trypsin solution in a given time interval was found to be the same as the amount of trypsin diffusing from the trypsin plus virus solution. Therefore, the rate of diffusion of trypsin is not changed by the presence of virus at the above mentioned concentration. This may be regarded as an indication that a combination of virus with trypsin does not occur, since the experimental conditions were most favorable for the combination. However, it cannot be considered absolute proof of the absence of such combination because of the lack of information concerning the size of the virus particle, the maximum number of particles present in a given virus solution and the probable ratio of virus to trypsin in such a complex.

DISCUSSION

The evidence obtained in this investigation indicates that the loss of infectivity of the virus of tobacco mosaic upon the addition of trypsin is not due to the proteolytic action of the enzyme. Tryptic hydrolysis is a time reaction which takes place rapidly only within a definite and fairly narrow range of hydrogen-ion concentration, whereas the loss of infectivity of the virus takes place immediately and over a wide range of hydrogen-ion concentration. Resynthesis or reactivation of the virus would not be expected if it were actually hydrolyzed, yet its infectivity in a trypsin-virus solution was regained by such simple procedures as dilution, heating, or removal of the trypsin from the solution by self-digestion or by digestion with pepsin. The fact that nonproteolytic substances, such as globin and trypsinogen, reduce the infectivity of the virus suggests that the reduction in the case of trypsin may not be due to proteolysis. No evidence from these experiments indicates that the virus of tobacco mosaic is a protein.

It was found possible to demonstrate the loss of infectivity of virus on the addition of trypsin, using several different viruses, and measuring the loss on many different species of plants. The fact that the rate of diffusion of trypsin was found to be unchanged in the presence of a fair concentration of virus is an indication that trypsin does not cause the loss of infectivity by forming an inactive complex with virus. Evidence that trypsin affects the plant and that the degree of its action on plants may vary from one species to another, was obtained when it was found possible to prepare a trypsin-virus solution that produced lesions on *Nicotiana glutinosa* and yet failed to produce lesions on *Phaseolus vulgaris*. Similar evidence was obtained in experiments in which it was found that addition of small constant amounts of trypsin to dilutions of virus caused a fairly constant decrease in the number of lesions, at all dilutions, when tested on *N. glutinosa*. The difference in the response of *N. glutinosa* and *P.*

vulgaris to the same trypsin-virus solution, and the fact that the degree of inhibition was fairly constant on the former when the actual amount of virus present was varied widely, is additional evidence that trypsin affects the plant. It is concluded, therefore, that the effect of trypsin in causing a loss in virus infectivity is due chiefly to its action on the plant. This conclusion is at variance with that of Caldwell (1) who reported that he found no action of trypsin on the tissues of the host plant. He found the average number of lesions, on leaves of *N. glutinosa* inoculated with virus, to be about the same, whether or not the leaves were rubbed with a trypsin solution previous to inoculation. In our experiments, which have been repeated several times, preliminary treatment with trypsin, either by rubbing or spraying, has always resulted in a marked decrease in the average number of lesions on inoculation with virus.

The action of trypsin on the test plant was found to be temporary, since the inhibitory effect of trypsin, which had been sprayed on a plant, was eliminated almost entirely by thoroughly washing the plant before inoculation with virus. Washing also decreased markedly the effect of trypsin that had been rubbed over a leaf. All the results indicate that trypsin must be present with the virus, either on the leaf at the time of inoculation, or within about thirty minutes thereafter in order to produce its inhibitory effect. Since it appears that this effect is caused by the action of trypsin on the plant, the infected cells seem to require at least 30 minutes to heal or transmit the virus to an adjacent uninjured cell, into which it may be assumed trypsin does not enter. If trypsin is present at the time of inoculation or within 30 minutes thereafter, it apparently affects infected cells, possibly by killing them, so that the virus does not spread and infect the plant. The results indicate, therefore, that the establishment of virus in a plant is not a rapid process, since infection may be inhibited by subsequent treatment. It appears to be, on the contrary, a time process requiring at least 30 minutes for completion.

SUMMARY

The decrease in infectivity of the virus of tobacco mosaic on addition of trypsin, as measured on *Nicotiana glutinosa* L. and *Phaseolus vulgaris* L., is not due to the proteolytic activity of the trypsin, because (1) the loss in infectivity is immediate, (2) it may take place over a wide range of hydrogen-ion concentrations, including some at which trypsin is inactive proteolytically, and (3) the infectivity of the virus may be regained by heat, by dilution, or by digestion and removal of trypsin.

A similar loss of the infectivity takes place on addition of trypsinogen or globin, proteins, which possess no proteolytic activity, but which, like trypsin, have isoelectric points above pH 7.

Virus, sufficiently concentrated so that it produces lesions on *Phaseolus vulgaris* when diluted 10^{-8}, does not affect the rate of diffusion of trypsin at a concentration of about 1 mg. of trypsin nitrogen per cubic centimeter. This indicates that there is no combination of virus with trypsin.

It is possible to prepare a trypsin-virus solution that will produce no lesions on plants of *Phaseolus vulgaris*, but will produce many lesions when tested on plants of *Nicotiana glutinosa* having about an equal susceptibility to untreated virus. This demonstrates that trypsin affects, in some way, the cells of plants of *P. vulgaris*.

The addition of a small constant amount of trypsin to dilutions of tobacco-mosaic virus causes a fairly uniform reduction in the number of lesions on *N. glutinosa* at such dilutions.

The decrease of the infectivity of this virus on addition of trypsin has been demonstrated upon a number of different species of plants including *Nicotiana glutinosa*, *N. acuminata* Hook., *N. langsdorffii* Schrank, *N. rustica* L. var. Winnebago, *N. tabacum* L. var. *purpurea* × *glutinosa*, *N. tabacum* var. Turkish, *Datura stramonium* L., *Phaseolus vulgaris* var. Early Golden Cluster, *Physalis angulata* L., *Solanum melongena* L. var. Black Beauty, *S. pseudo-capsicum* L., and *Capsicum frutescens* L.

Other viruses whose action is similarly affected by trypsin are a masked and a yellow strain of tobacco mosaic, aucuba mosaic, severe etch, tobacco ring spot, ordinary cucumber mosaic, and a yellow strain of cucumber-mosaic virus.

Trypsin sprayed or rubbed on the leaves of plants of *Nicotiana glutinosa*, *N. tabacum* var. Turkish, and *Phaseolus vulgaris* markedly lowers their susceptibility to virus. This effect is temporary, however, since the plants become susceptible to the virus again upon washing trypsin from the leaves with water.

The inhibitory effect of trypsin may be demonstrated if it is administered by either rubbing or spraying within about 30 minutes after the inoculation of the leaf with virus. This indicates that the establishment of virus in a plant, so that it may not be interrupted by subsequent treatment, is not instantaneous, but requires at least 30 minutes for completion.

As a whole, the results indicate that the loss of infectivity upon the addition of trypsin to a virus solution is due chiefly to the action of trypsin on the plant.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

LITERATURE CITED

1. CALDWELL, JOHN. The physiology of virus diseases in plants. Ann. Appl. Biol **20**: 100–116. 1933.
2. HOLMES, F. O. Local lesions in tobacco mosaic. Bot. Gaz. **87**: 39–55. 1929.
3. ————. A masked strain of tobacco-mosaic virus. Phytopath. **24**: 845–873. 1934.
4. JENSEN, JAMES H. Isolation of yellow-mosaic viruses from plants infected with tobacco mosaic. Phytopath. **23**: 964–974. 1933.
5. LOJKIN, MARY, and C. G. VINSON. Effect of enzymes upon the infectivity of the virus of tobacco mosaic. Contrib. Boyce Thompson Inst. **3**: 147–162. 1931.
6. MATSUMOTO, TAKASHI, and KÔETSU SOMAZAWA. Immunological studies of mosaic diseases. I. Effect of formolization, trypsinization and heat-inactivation on the antigenic properties of tobacco-mosaic juice. Jour. Soc. Trop. Agric. **2**: 223–234. 1930. *Ibid.* **3**: 24–33. 1931.
7. NORTHROP, J. H., and M. L. ANSON. A method for the determination of diffusion constants and the calculation of the radius and weight of the hemoglobin molecule. Jour. Gen. Physiol. **12**: 543–554. 1929.
8. ————, and M. KUNITZ. Crystalline trypsin. I. Isolation and tests of purity. Jour. Gen. Physiol. **16**: 267–294. 1932.
9. PRICE, W. C. Local lesions on bean leaves inoculated with tobacco-mosaic virus. Amer. Jour. Bot. **17**: 694–702. 1930.
10. ————. Isolation and study of some yellow strains of cucumber mosaic Phytopath. **24**: 743–761. 1934.
11. SAMUEL, GEOFFREY, and J. G. BALD. On the use of the primary lesions in quantitative work with two plant viruses. Ann. Appl. Biol. **20**: 70–99. 1933.
12. SCHERP, HENRY W. The diffusion coefficient of crystalline trypsin. Jour. Gen. Physiol. **16**: 795–800. 1933.
13. TAKAHASHI, WILLIAM N., and T. E. RAWLINS. Electrophoresis of tobacco-mosaic virus. Hilgardia **4**: 441–463. 1930.
14. VINSON, C. G. Possible chemical nature of tobacco-mosaic virus. Science n.s. **79**: 548–549. 1934.
15. ————, and A. W. PETRE. Mosaic disease of tobacco. Bot. Gaz. **87**: 14–38. 1929.
16. ————, and ————. Mosaic disease of tobacco. II. Activity of the virus precipitated by lead acetate. Contrib. Boyce Thompson Inst. **3**: 131–145. 1931.

THE WEDGE GRAFT AS A MEANS OF CONTROLLING OVERGROWTHS AT THE UNION OF NURSERY APPLE TREES[1]

R. F. SUIT

(Accepted for publication December 6, 1933)

When it was established through the work of Riker and Keitt (5, 6) and Muncie (3) that about 95 per cent of the overgrowths at the union of grafted apple trees is not due to *Pseudomonas tumefaciens* E. F. Sm. and Town., the problem of controlling these malformations became simplified. Riker and Muncie (8) showed that well-matched and fitted grafts developed less callus knot than poorly fitted ones. In 1928, Melhus, Muncie, and Fisk (2) suggested the wedge graft as being beneficial in the control of callus knot. In field trials the same year Muncie (4) obtained an increase of about 20 per cent in clean trees by the use of the wedge graft. Later work by Riker, Keitt, and Banfield (7) showed that a light grade of adhesive plaster or nurseryman's tape applied as a wrapper at the union of apple grafts also reduced the amount of callus knot by about 20 per cent. In 1929 Maney (1) reported that the double tongue graft controlled callus knot on Sharon apple.

In view of the new understanding referred to above regarding the causation of overgrowths at the union of apple grafts, coupled with the findings of Melhus, Muncie, and Fisk (2) and Muncie (4), that wedge grafts showed some tendency to reduce the amount of callus knot, a careful study of the comparative value of the different types of grafts from the standpoint of stand of trees, strength of graft unions, and location of and freedom from callus knot was warranted.

METHODS AND MATERIALS

The comparative value of wedge or cleft and single-tongue or whip grafts for the control of callus knot was studied in 7 different nurseries,[2] while the value of the double-tongue graft was studied in 2 of the nurseries.

[1] The writer wishes to acknowledge his indebtedness to Dr. I. E. Melhus of the Botany Department of Iowa State College for suggesting the problem and for criticism and suggestions received throughout the course of these investigations and the preparation of this manuscript. Credit also is due Dr. J. H. Muncie, now of the Botany Department of Michigan State College, for assistance in planning some of the field work.

[2] The writer is indebted to the following nurseries for excellent cooperation in the study of this problem: Mount Arbor Nurseries and Shenandoah Nurseries, Shenandoah, Iowa; Plumfield Nurseries, Fremont, Nebraska; Marshall Nurseries, Arlington, Nebraska; J. H. Skinner and Company, Topeka, Kansas; Stark Bros. Nurseries, Stigler, Oklahoma; and Hillenmeyer Nurseries, Lexington, Kentucky.

Two of these nurseries are in Iowa, 2 in Nebraska, and 1 each in Kansas, Oklahoma, and Kentucky. In each case the plantings were made in the center of a large block of apple trees, so that the cultivation and treatment would be representative of the usual nursery practice. Scions of the Wealthy apple generally were used because of the susceptibility of this variety to callus-knot formation. However, several other varieties were included at various times.

The wedge graft is commonly used for top grafting and double working of apple trees, the side graft being a modified wedge graft. In piece-root grafting the wedge graft is easily made and requires one less cut than the whip graft. The usual procedure in making the wedge graft is somewhat as follows: The base of the scion is cut to a feather-edge wedge, $\frac{3}{4}$ inch long, by 2 cuts, as shown in figure 1, A, *a*. In the case of the stock, a slightly cross-grained cleft is made to a depth of about $\frac{1}{8}$ inch deeper than the length of the scion wedge (Fig. 1, A, *b*). The cleft is slightly cross-grained to prevent splitting. In making the graft, the wedge is fitted tightly into the cleft of the stock, so that the cut surface of the scion is completely covered by the stock. Figure 1, B and C, shows a properly fitted graft.

The cambium of the scion wedge should be matched along one side with that of the stock, if the scion is smaller in diameter. The graft may be wrapped with waxed string or with nurseryman's tape as recommended by Riker, Keitt, and Banfield (7). It often happens that the wedge graft is improperly made. Figure 1, D, shows 2 common errors. In the one the scion wedge (*d*) is larger in diameter than the stock, which allows the cambium along one side of the wedge and the scion tip to projejct beyond the stock and thus give favorable conditions for callus-knot development, as reported by Riker and Muncie (8) and Sass (9). In the other the cut surface (*c*) of the scion is not completely covered by the stock, and knots may develop from this exposed cut surface.

The double-tongue graft recommended by Maney (1) is similar to the whip or single-tongue graft. The tip of the scion lip is wedged by removing about $\frac{1}{4}$ inch of bark and wood from the outer side. This wedged scion lip is inserted into a second cut that is made in the stock. This gives 2 tongues fitting into the stock, hence the name double-tongue graft. Two more cuts are required for this graft than for the whip graft.

The grafts used in the experimental plots were made at each nursery where the plots were located. The making of the grafts was supervised by J. H. Muncie or by the writer. In planning an experiment, an equal number of both wedge and whip grafts was used for each variety. After these grafts were made they were stored and planted in accordance with the established practice in that particular nursery. No special care was given the

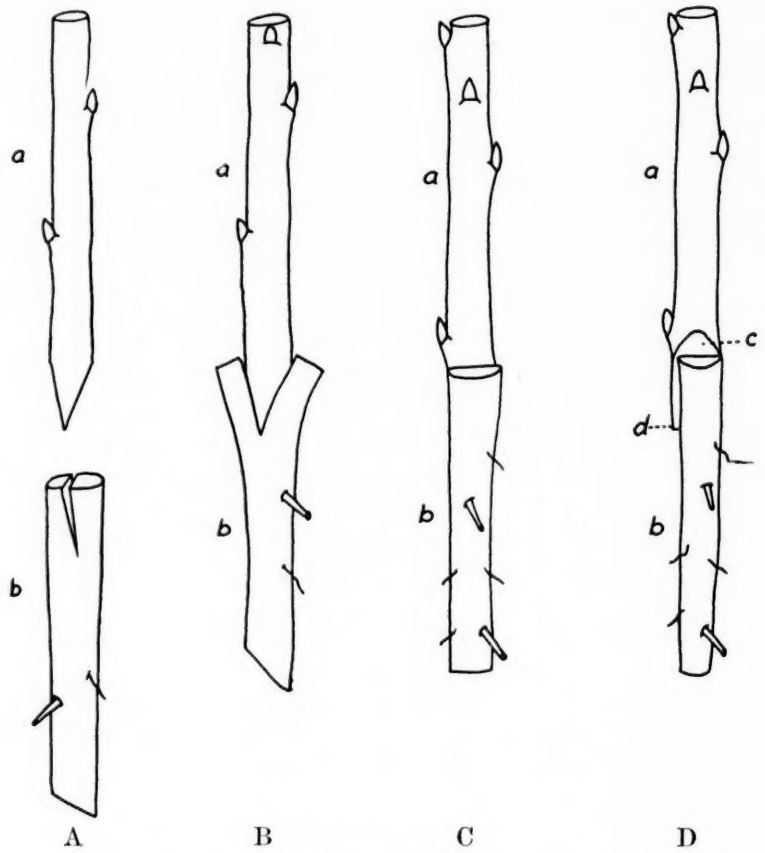

FIG. 1. A–D. The wedge or cleft graft, as used in piece-root grafting. In D, *c* and *d*, are shown two common errors in fitting.

experimental grafts throughout the growing season. Final examination of the grafts was made at digging time, when the trees were 2 years old. In several cases the grafts were examined when the trees were only a year old.

A COMPARISON OF THE RIGIDITY OF THREE TYPES OF GRAFTS

Rigidity in apple grafts is important in nursery practice, especially with reference to handling, planting, and various methods of cultivation. To determine, if possible, the relation between the strength of the different types of grafts, 600 Wealthy apple grafts were obtained from each of 3 nurseries. These grafts were of the 3 types, wedge, whip, and double-tongue. An equal number of each type of graft was wrapped with waxed string and nurseryman's tape, following which all grafts were allowed to callus for a month. The rigidity of the union of the grafts was determined

when held in different positions. The graft was suspended by supporting it 1 inch on either side of the union, while the force was applied at the union.

The apparatus[3] used for determining the rigidity of the grafts was devised for testing the strength of corn stalks. By means of the apparatus, breaking pressure was brought upon the graft; the stress or tension was recorded by means of a calibrated spring. The breaking force in pounds for the grafts of various sizes in the different lots is given in table 1. Each figure given is an average of the results from at least 10 grafts and, in many sizes, as many as 25 or 30. The findings show that more force was required to break grafts of greater diameter. An average of the measurements for each lot shows that the grafts wrapped with waxed string required about the same force for breaking, regardless of the type of graft. The grafts usually broke more readily parallel to the cut of the graft. The mechanical strength of grafts wrapped with nurseryman's tape was increased about 3 pounds. It is shown also that the uncut scion of the same diameter was stronger than the graft, whether wrapped with waxed string or with nurseryman's tape. These grafts were not made especially for this test and were the usual bench run at the nursery. In observing the individual grafts as they were broken it was seen that twice the force often was required to break the better made and fitted grafts as compared with the poorly fitted grafts of the same size.

THE STAND OF APPLE TREES OBTAINED FROM THREE TYPES OF GRAFTS

One of the important problems of the nurseryman is to obtain from the grafts planted a high percentage stand of clean trees. Table 2 shows the percentage of trees obtained after 2 seasons' growth, as well as the percentage of clean trees obtained from the original lot of 500 grafts in each case. These data are from trees dug from the experimental plots during the fall of 1930. Complete figures for 1928 and 1929 are not available, but, in general, agree with the results shown in table 2. Nearly all factors shown affect the stand of trees. The greatest difference in stand seems to be the result of location. Grafts grown in Kansas produced a much better stand than those in Iowa and Nebraska; but there is less difference between total trees and clean trees from grafts grown in Nebraska. The type of graft does not seem materially to affect the stand, although the wedge graft may show a slight advantage. Usually, the type of wrapper is not important with reference to stand; the variations one way or another are not constant.

While table 2 shows the final stand obtained from the growing of apple grafts, it does not indicate the reaction of the various types of grafts during

[3] The writer is indebted to Dr. L. W. Durrell, formerly of the Botany Department, Iowa State College, for the original design of the apparatus used.

TABLE 1.—*A summary of the force in pounds necessary to break wedge, whip, and double-tongue-grafts when wrapped with waxed string or nurseryman's tape*

Lot	Type of graft and treatment	Scion caliper in sixteenths of an inch														
		2–3				3–4				4–5				5–6		
		Stock caliper in sixteenths of inches														
		2–3	3–4	4–5	5–6	3–4	4–5	5–6	6–7	4–5	5–6	6–7	7–8	5–6	6–7	Av.
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
I[a]	Whip graft, string wrapped		5.5	9.2		7.5	10.4	12.1	15.0	10.7	17.9	12.6	18.5	12.0	18.0	12.4
II[b]	" " " "		6.4	10.5		8.2	10.3	13.1		12.4	14.4	17.1	24.5	19.5	19.0	14.1
III[a]	" " tape wrapped	5.5	7.4	8.9		11.5	13.1	14.5		21.8	23.7	25.2		22.0	29.0	16.8
IV[b]	" " " "	5.5	8.0	10.0	11.0	10.4	12.3	14.3		17.8	19.6	26.0		25.4	25.0	15.5
V[a]	Wedge graft, string wrapped	6.0	6.1	6.3		8.2	10.5	11.4		11.6	13.3	14.7	17.5	21.0	19.5	12.2
VI[b]	" " " "		6.7	6.3		7.7	9.8	12.8	16.5	15.6	18.0	19.5		19.0	22.0	13.9
VII[a]	" " tape wrapped		7.6	8.7	9.5	8.9	12.4	13.6	17.2	16.5	21.0	24.5	28.5	29.0	29.0	17.4
VIII[b]	" " " "		6.5	7.2		10.4	10.5	12.4	12.7	16.5	22.1	23.8	20.5	25.3	25.6	16.1
IX[a]	Double-tongue graft, string wrapped		6.0	7.0	8.0	8.5	9.4	11.1	14.0	15.1	17.8	23.3	18.5	16.9	25.5	14.3
X[b]	" " " "		6.1	7.3		7.1	9.7	11.4		12.8	13.5	15.5		16.3	20.2	12.4
XI[a]	" " tape wrapped		6.2	7.6	8.5	8.9	10.5	11.9	17.0	17.3	19.3	21.2		24.5	26.8	15.9
XII[b]	" " " "		6.0	7.3		9.9	11.3	13.4	12.05	14.6	18.5	21.5		20.4	24.2	15.7
	Scion		8.5				14.0			23.5				30.0		19.0

[a] Grafts broken parallel to the cuts of the grafts.
[b] Grafts broken at right angles to the cuts of the grafts.

TABLE 2.—*Effect of type of graft and kind of wrapper on the stand of two-year-old apple trees*

Variety	Location	Wedge graft				Whip graft			
		Tape wrap		String wrap		Tape wrap		String wrap	
		Stand	Clean trees	Stand	Clean trees	Stand	Clean trees	Stand	Clean trees
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Wealthy	Iowa	94[a]	93	62	59	87	85	82	78
"	Nebraska	74	71	67	64	70	64	68	59
"	Kansas	98	87	96	68	96	76	97	55
Yellow transparent	Nebraska	67	64	79	76	82	75	76	68
Yellow transparent	Kansas	99	88	99	68	96	80	94	65
Whitney	Nebraska	78	78	78	76	63	58	76	62

[a] Percentage of stand obtained from planting 500 grafts.

the first season's growth. For this work, each of the three types, whip, wedge, and double-tongue, were divided into 4 series as follows:

1. Wrapped with tape; scion $\frac{3}{16}$ inch and less in diameter.
2. Wrapped with tape; scion greater than $\frac{3}{16}$ inch in diameter
3. Wrapped with string; scion $\frac{3}{16}$ inch and less in diameter.
4. Wrapped with string; scion greater than $\frac{3}{16}$ inch in diameter.

These grafts were planted on April 25, 1930, in the experimental plots at Ames, Iowa. Stand counts were made each month throughout the summer, and on October 25 the trees were dug and examined. The summer of 1930 being extremely dry, the results obtained from this study would indicate the performance of the 3 types of grafts under adverse conditions. More favorable conditions were obtained in the plots in other localities the previous year, the results of which have been given. Table 3 shows the

TABLE 3.—*Results of stand counts of Wealthy apple grafts at intervals of one month throughout the summer of 1930*

Date counted	Double-tongue series				Whip series				Wedge series			
	1	2	3	4	1	2	3	4	1	2	3	4
4/25/30	262	289	292	275	291	261	180	324	266	289	248	307
5/31/30	223	244	259	241	247	184	157	300	157	222	208	268
6/30/30	201	227	245	241	205	183	157	283	157	222	202	259
8/4/30	161	171	183	175	191	183	141	255	138	174	181	236
9/8/30	159	167	177	167	190	182	141	254	137	174	179	235
10/6/30	157	165	177	167	185	179	141	252	137	174	177	235
Stand percentage of grafts planted	59.9	57.1	60.6	60.7	63.5	68.5	78.3	77.7	51.5	60.2	71.3	76.5
Percentage of final stand, clean trees	86.6	84.2	61.0	47.8	83.7	89.3	60.9	49.2	92.7	92.5	73.4	74.8

stand throughout the summer as well as the number of clean trees obtained in each type of graft. The double-tongue graft shows the lowest final stand in all series, with the exception of series 1 of the wedge graft. When the percentage of clean trees produced is considered, the double-tongue graft was also the lowest. The type of wrapper did not materially affect the stand of the double-tongue grafts, although the grafts wrapped with tape gave a higher percentage of clean trees. The whip graft showed the highest percentage of stand, while the clean trees produced exceeded the double-tongue graft by at least 10 per cent in all but series 1. The grafts wrapped with tape gave a higher percentage of clean trees, although the string-wrapped grafts gave a higher final stand. The wedge graft was between the other 2 types of grafts in the stand produced. The percentage of clean trees produced from grafts wrapped with tape was lower in series 1 than

the other 2 types. In series 2 the percentage was above the double-tongue and below the whip graft. Those grafts wrapped with string showed a much higher percentage of clean trees than either of the other 2 types. As was found in the whip grafts, the wedge grafts wrapped with string showed a higher percentage of stand than those wrapped with tape.

In nursery practice the grafts usually are not counted when being planted. The percentage of clean trees is then calculated from the final stand. As shown in table 3, when the percentage of clean trees is calculated from the final stand, the wedge graft wrapped with tape are found to be over 90 per cent clean trees, while those grafts wrapped with string showed over 70 per cent of clean trees. In every case these percentages are higher than those obtained from the double-tongue or whip grafts, while in the case of the string-wrapped grafts the increase shown by the wedge graft is over 10 per cent in series 3 and over 20 per cent in series 4.

The size of the scion used for grafting did not materially affect the stand of trees produced, except in the wedge grafts, where the larger scions produced a higher percentage of trees. However, in the case of series 4 of the double-tongue and whip grafts, the smaller scions showed at least 10 per cent more clean trees than the grafts made with the larger scions. This fact was not apparent in the wedge grafts.

During the first month of the growing period the grafts are becoming established. Table 3 shows that the greatest loss in stand of wedge and whip grafts occurred during this period. A second rapid loss during the third month was shown by the double-tongue grafts. After the third month of growth there was practically no further loss in stand. During 1930 the third month, July, was extremely dry. This might account for the rapid drop during this period. Under normal condiitons this second drop in stand might not occur and, after the initial loss, the stand would tend to remain constant.

RESULTS OF THE USE OF THE WEDGE GRAFT FOR THE CONTROL OF CALLUS KNOT

Preliminary trials by Melhus, Muncie, and Fisk (2) and Muncie (4) showed that the wedge graft was beneficial in the control of callus knot. Consequently, numerous experiments at the 7 nurseries were conducted in 1928, 1929, and 1930. A summary of the results obtained when wedge grafts of the different varieties of apple were made is shown in table 4. These results show that in every case a higher percentage of clean trees was obtained by using the wedge graft. The greatest increase in clean trees was obtained with Wealthy, Yellow Transparent, Sweet Russet, Whitney, and Duchess varieties. On the other hand, Grimes Golden, Delicious, Jonathan, and Stayman usually gave over 80 per cent clean trees, whether

TABLE 4.—*A comparison of the percentage of clean trees obtained from string- and tape-wrapped wedge and whip grafts*

Variety	1928					1929									
	String-wrap					String-wrap					Tape-wrap				
	Wedge		Whip		Increase	Wedge		Whip		Increase	Wedge		Whip		Increase
	No.	Clean	No.	Clean		No.	Clean	No.	Clean		No.	Clean	No.	Clean	
		Per cent		*Per cent*	*Per cent*		*Per cent*		*Per cent*	*Per cent*		*Per cent*		*Per cent*	*Per cent*
Wealthy	530	74	348	55	19	2091	73	1601	55	18	501	90	434	83	7
Yellow Transparent	509	72	124	68	14	894	74	498	53	21					
Sweet Russet	1252	94	1362	66	28										
Whitney	362	91	348	67	24	1308	88	865	71	17					
N. W. Greening	640	75	857	62	13	831	89	658	82	7					
Jonathan	520	82	459	76	6										
Grimes Golden	227	91	101	85	6	715	88	278	85	3					
Delicious						380	94	175	93	1					
Duchess						532	76	581	54	22					
Total	4040	84	3599	66	18	6751	81	4656	65	16	501	90	434	83	7

TABLE 4.—(*Continued*)

Variety	1930									
	String-wrap					Tape-wrap				
	Wedge		Whip		In-	Wedge		Whip		In-
	No.	Clean	No.	Clean	crease	No.	Clean	No.	Clean	crease
		Per cent		*Per cent*	*Per cent*		*Per cent*		*Per cent*	*Per cent*
Wealthy	958	82	1089	75	7	1140	94	1104	88	6
Yellow Transparent	695	77	662	75	2	673	91	688	87	4
Sweet Russet										
Whitney	196	96	190	81	15	196	99	157	92	7
N. W. Greening										
Jonathan										
Grimes Golden										
Delicious										
Duchess										
Total	1849	80	1941	75	5	2009	93	1949	87	6

tape or string-wrapped wedge or tongue grafts were employed. In 1928, as shown in Table 4, the wedge graft increased the percentage of clean trees by 18 per cent and in 1929 by 16 per cent, while in 1930 a difference of only 5 per cent was found. When the tape wrapper was used, the wedge graft still showed 6 or 7 per cent increase in clean trees over the whip graft. It is to be noted that the percentage of clean trees in the whip grafts wrapped with string is higher in 1930 than in 1928 and 1929. This might be accounted for either by the fact that the nurserymen were exercising more care in the making of the grafts or by the dry season.

Several experiments were conducted in which the double-tongue graft also was used. Table 5 shows a summary of the results obtained by the

TABLE 5.—*Results of the use of the double-tongue graft on the control of callus knot*

Variety	Year	Wrapper	No. trees	Percentage clean trees
N. W. Greening	1928	Raffia	961	60.4
Wealthy	1928	"	900	42.5
"	1928	String	1210	64.8
"	1930	"	344	54.9
"	1930	Tape	322	85.4

use of this type of graft. In only one case did the double-tongue produce a higher percentage of clean trees than the whip graft. This was on Wealthy in 1928. The data show that at no time was there any question as to the superiority of the wedge graft for the control of callus knot when compared to the double-tongue graft. The use of nurseryman's tape as a wrapper

was also beneficial in the case of the double-tongue graft but not to such an extent as to give better results than the other 2 types of grafts.

Since there is a considerable difference between the growing season in Iowa and that of various other apple-growing sections, it is possible that this fact might influence the amount of callus knot found in the various localities. Table 6 shows the precipitation and temperature for certain

TABLE 6.—*A summary of certain meteorological data for certain localities in the States of Oklahoma, Kansas, Kentucky, Nebraska, and Iowa (10)*

State	Precipitation	Average temperature for 20 years		
		Mean annual	Normal	
	Annual		March	November
	Inches	° *F.*	° *F.*	° *F.*
Oklahoma	31.15	59.4	50.0	48.8
Kansas	37.65	55.3	44.5	44.1
Kentucky	43.3	55.0	43.7	44.8
Nebraska	27.7	50.6	37.0	38.5
Iowa	32.04	49.5	35.9	38.4

points that are nearest to the nursery sections in each of the 5 States. It is noted that the temperatures for March and November are higher in Oklahoma, Kansas, and Kentucky, which would give a longer period of growth in those States. There also is some difference in the annual precipitation of Kansas and Kentucky and Oklahoma, Nebraska, and Iowa. A summary of the data from all the apple trees examined in the 5 different States for the 3-year period is shown in table 7. This table shows that the smallest percentage (58 per cent) of clean trees produced from the wedge graft, was found in Oklahoma, while, in Kansas, 69 per cent clean trees were found. In Iowa 83 per cent was found and in Nebraska 86 per cent. From the trees examined, Kentucky showed the highest percentage of clean trees. However, when the grafts were wrapped with tape, as indicated by results from three States, these States still held their rank, while the percentage of clean trees increased by 10 per cent or more.

Although the percentage of clean trees produced from the different types of grafts is important, it also is advantageous to know where the callus knots were produced on the affected trees. This information would be of value in the making of grafts so that care could be taken to avoid those practices that are apt to cause callus knot. A summary of the location of callus knots found on the 3 types of grafts is shown in table 8. The whip

TABLE 7.—*Summary of three years' results, showing the effect of the geographical location on the percentage of overgrowths found at the union of piece-root-grafted apple trees*

Location	Totals									
	String-wrap					Tape wrap				
	Wedge		Whip		Increase	Wedge		Tape		Increase
	No.	Clean	No.	Clean		No.	Clean	No.	Clean	
		Per cent		*Per cent*	*Per cent*		*Per cent*		*Per cent*	*Per cent*
Oklahoma	739	58	1000	68	−10					
Kentucky	682	96	783	94	2					
Kansas	1412	69	1488	61	8	997	87	961	81	6
Nebraska	9909	86	5505	78	8	725	97	697	93	4
Iowa	4247	83	4871	63	20	1776	96	871	90	6
Total	16,989	83	13,647	71	12	3498	93	2529	87	6

graft shows the highest percentage of callus knots on the scion lip and the side of the union. In the case of the double-tongue graft the callus knots at the scion lip have been reduced because the scion lip is inserted into a second cut in the stock. However, there are more cuts exposed on the side of the union and, as indicated in table 8, the majority of the callus knots occur at this point. The wedge graft shows a distribution of callus knots in 3 places, scion lip, side of the union, and exposed scion above the union. The callus knots produced at the scion lips may be due to 2 factors: firstly, the scion is too large for the stock as shown in figure 1, D, *d*, or, secondly, the

TABLE 8.—*A summary of the location of callus knots found on string-wrapped whip, wedge, and double-tongue grafts*

Type of graft	Knots examined	Location of callus knots				
		Scion lip	Stock lip	Side union	Exposed scion above union	Shoulder of stock
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Whip	4775	35.8[a]	10.8	52.1	1.3	0.0
Double-tongue	1312	8.5	8.7	82.8	0.0	0.0
Wedge	2152	29.2	0.0	40.7	19.4	10.7

[a] Percentage of knots examined.

scion is broken over in cultivation, principally by the practice of "hooking" for the removal of weeds, so that the scion lip projects out beyond the stock. The callus knots produced at the side of the union might be remedied by a more even cut and a closer fitting of the cambium of scion and stock. The callus knots on the exposed scion above the union (Fig. 1, D, *c*) can be eliminated by inserting the scion into the stock until the cut surface is covered. All 3 types of grafts show about the same percentage of callus knots produced from the upper portion of the stock.

Some objections to the use of the wedge graft have been made because of the reported tendency (1) toward the development of sprouts from the graft shoulder. However, in all of the thousands of wedge-grafted trees observed, no such sprouts were found when the trees were 2 years old.

DISCUSSION

It is evident from the results presented that the wedge graft, whether wrapped with string or nurseryman's tape, gives a higher percentage of clean trees than the whip or double-tongue grafts. Tests of the breaking strength of the grafts show that when properly made and handled, the wedge graft is as strong as the whip or double-tongue grafts. Stand counts have shown that the difference in percentage of stand between the wedge and whip grafts is small, one type of graft showing perhaps 5 per cent better on one variety one year, and the next year, or in another locality, showing no difference, or the relationship may be reversed. Callus knots were found on the wedge graft in 3 locations: scion lip, side of the union, and exposed scion above the union. A remedy for these callus knots has been suggested. The use of nurseryman's tape largely prevents the formation of excess callus by covering the wounded tissues. This is indicated by the fact that an increase in clean trees of over 10 per cent is obtained in most cases. The evidence presented here justifies the recommendation of the wedge graft, wrapped with tape, for the control of overgrowths at the graft union.

It is noted that the precentage of overgrowths varies somewhat according to the variety. The wedge graft is especially recommended for the more readily callusing varieties, such as Wealthy, Yellow Transparent, and Duchess. Table 6 shows that the greatest increase in percentage of clean trees, due to the use of the wedge graft, was on those more readily callusing varieties, while on the more slowly callusing varieties such a marked increase was not present. However, it should be noted that such varieties as Jonathan, Delicious, and Grimes Golden showed rather high percentages of clean trees, even when the whip graft was used. An increase of about 5 per cent in clean trees was obtained by the use of the wedge graft on this group of varieties.

SUMMARY

A study of the mechanical strength of wedge, whip, and double-tongue grafts wrapped with string showed no significant differences. When the grafts were wrapped with nurseryman's tape, the strength was increased about 3 pounds regardless of the type of graft. A well-fitted graft is twice as strong as a poorly fitted one of the same size.

No marked difference was found between the stand obtained from wedge and whip grafts. From a limited number of data, the double-tongue graft showed a lower percentage of stand than the wedge and whip grafts. The kind of wrapper used, whether string or tape, did not have any significant effect upon the percentage of stand except in the case of the wedge and whip grafts during a dry season, when the tape wrapper showed a 10 per cent lower stand. Under the droughty conditions of the summer of 1930 the tape-wrapped wedge and whip grafts showed some increase in stand when scions of a caliper of over $\frac{3}{16}$ inch were used. Other results with respect to caliper of scion showed no material differences in the percentage of stand obtained.

An increase in the percentage of clean trees was obtained on Wealthy, Yellow Transparent, Sweet Russet, Whitney, Northwestern Greening, Jonathan, Grimes Golden, Delicious, and Duchess varieties by using the wedge graft. Wealthy, Yellow Transparent, Whitney, and Duchess were varieties that showed the greatest increase from the use of the wedge graft. The least increase was obtained on Jonathan, Grimes Golden, and Delicious. The greatest increase obtained by the use of the wedge graft was 28 per cent on Sweet Russet in 1928.

The length of the growing season seemed to have an effect on the amount of callus knot, as a lower percentage of clean trees was obtained from grafts grown in Oklahoma and Kansas in comparison with the results obtained in Nebraska and Iowa.

The use of nurseryman's tape as a wrapper increases the percentage of clean trees about 10 per cent, whether wedge or whip grafted. The greatest increase due to wrapping with tape was 28 per cent on Wealthy whip grafts in 1929. The least increase was 3 per cent on Whitney wedge grafts in 1930.

The larger percentage of callus knots found on the wedge graft occurred on the scion lip, side of the union, and the exposed cut-surface of the scion above the union. Proper fitting and handling of the wedge graft would eliminate most of these knots.

MACDONALD COLLEGE, QUEBEC, CANADA.

LITERATURE CITED

1. MANEY, T. J. Controlling crowngall on apple trees by an improved method of root grafting. Iowa Hort. Soc. Rept. **64**: 23–26. 1929.
2. MELHUS, I. E., J. H. MUNCIE, and V. C. FISK. Grafting as a further means of preventing callus knots on apple. Phytopath. **18**: 127–128. 1928. (Abst.)
3. MUNCIE, J. H. A study of crowngall caused by *Pseudomonas tumefaciens* on Rosaceous hosts. Iowa State Coll. Jour. Sci. **1**: 67–117. 1926.
4. ————. Present status of the crowngall investigations. Iowa Hort. Soc. Rept. **63**: 204–206. 1928.
5. RIKER, A. J., and G. W. KEITT. Crowngall in relation to nursery stock. Science n.s. **62**: 184–185. 1925.
6. ————, ————. Studies of crowngall and wound overgrowth on apple nursery stock. Phytopath. **16**: 765–808. 1926.
7. ————, ————, and W. M. BANFIELD. A progress report on the control of crown gall, hairy root, and other malformations at the unions of grafted apple trees. Phytopath. **19**: 483–486. 1929.
8. ———— and J. H. MUNCIE. Suggestions on the preparation of apple grafts. Crop Prot. Inst. Bul. 9. 1926.
9. SASS, J. E. Formation of callus knots on apple grafts as related to the histology of the graft union. Bot. Gaz. **94**: 364–380. 1932.
10. U. S. Dept. of Agriculture, Yearbook of Agriculture, **1930**: 1054–1057. 1930.

CONIDIAL PRODUCTION IN SPECIES OF CERCOSPORA IN PURE CULTURE[1]

CLATUS M. NAGEL[2]

(Accepted for publication December 29, 1933)

Considerable difficulty was encountered in obtaining conidial production in pure culture in connection with a study of the host range of *Cercospora beticola* Sacc. The lack of abundant conidial production in pure culture with which to perform cross inoculations made it necessary to develop a method for obtaining abundant sporulation. Such a method has been found for the species of Cercospora studied. The effect of different media and a procedure for maintaining cultures in a sporulating condition are described in this paper.

Those who have investigated this problem present conflicting evidence regarding conidial production of not only *Cercospora beticola* but also other species of Cercospora. Duggar (3), working with *C. apii* Fres., in 1897, obtained conidia in pure culture when he grew the fungus on celery petioles and bean stems. The same investigator (4), however, in 1899, failed to obtain conidia in culture with *C. beticola.* He maintained cultures in the laboratory under various conditions over a period of 2 years, and reports that no type of fruiting was observed during his investigations. Klotz (9) reported conidial production with *C. apii* when he grew the fungus on celery refuse. Solheim (16) studied several species and was successful in obtaining conidia with only *C. ampelopsidis* Peck and *C. illinoensis* Barth.

Garman (5) reports that *Cercospora brunkii* Ell. & Galw. grew well on several media, but that it did not readily produce conidia. Jones and Pomeroy (8), McKay and Pool (10), Rand (13), Welles (19), and Wolf (20) reported similar difficulties, though other investigators were more successful. Aderhold (1) obtained conidia with *C. cerasella* Sacc., Coons and Larmer (2), working with *C. beticola,* observed conidia, produced somewhat sparingly over the entire mycelial mat, but in greater numbers at the borders of the colonies. They grew the cultures on a sugar-beet-leaf medium.

Hopkins (6) attributed his success in conidial production to a partial drying out of the potato-dextrose agar in plate cultures of *Cercospora medicaginis* Ell. & Ev. Nagel and Dietz (12) and Nagel (11) reported

[1] Journal paper No. J138 of the Iowa Agricultural Experiment Station, Ames, Iowa. Botany and Plant Pathology Section Project No. 76.

[2] The writer wishes to express his appreciation for the helpful advice and criticism given by Dr. S. M. Dietz during the course of the investigational work and the preparation of this manuscript.

abundant conidial production with *C. beticola, C. davisii* Ell. & Ev., *C. physalidis* Ell., *C. setariae* Atk., and *C. zebrina* Pass.

Schmidt (14) reports that *Cercospora beticola* seldom produces conidia in pure culture, and when it does, only sparingly. He obtained best results upon placing the cultures at temperatures of 25° C. for a short period and then at a cooler temperature of 15° C. He found one culture, among many, that had been held under ordinary summer temperatures for several months, that contained numerous conidia. He was unable to offer any explanation for this particular case. Stolze (17) also stated that he was unable to observe sporulation while studying *C. beticola* in pure culture.

Jenkins (7) reported that typical conidia of *Cercospora cerasella* have been produced in pure cultures from conidia and ascospores from this fungus. However, he states further that when conidia were planted on such media as potato-dextrose, corn-meal agar, bean and dextrose agar, synthetic nutrient agar, or cherry-leaf-decoction agar to which were added fragments of diseased leaves as an additional source of nutrients, no fruiting was observed, although the fungus made a good vegetative growth. If, however, tubes of these media on which the organism had been growing for a time were again sterilized and planted with conidia, conidia were produced. Welles (18) dealing with several Philippine species of Cercospora obtained conidia in a few days in pure culture. However, Welles (19) in earlier studies with *C. melongenae* Welles, in which he kept certain cultures under observation for 5 weeks observed no fruiting. Singh (15) was successful in obtaining conidia in pure culture with *C. patouillardi* Sacc., *C. leucosticta* Ell. and a *Cercospora* sp., but failed to obtain conidia in the case of a fourth species, *C. feuilleauboisii* Sacc.

PRELIMINARY EXPERIMENTS AND SOURCES OF CULTURES

The species of Cercospora used in the preliminary experiments were *C. beticola, C. davisii, C. dubia* (Riess) Wint., and *C. zebrina.* These were collected by S. M. Dietz and E. F. Vestal in the sugar-beet growing districts of northern Iowa. The isolations were made in the Botany and Plant Pathology laboratories at Ames, in the fall of 1930. These cultures were grown and maintained on potato-dextrose agar slants by E. F. Vestal until February 1, 1931. At that time transfers were obtained from each species for use in the following investigations.

In the preliminary trials the transfers were held under ordinary laboratory conditions from February, 1931, to August, 1931. During this time 30 consecutive mycelial transfers were made on potato-dextrose and sugar-beet-leaf agar slants. Microscopic examinations of the cultures were made in approximately 2 weeks from the date of transfer, when the fungus had produced a mycelial mat about 2 to 3 centimeters in diameter, but no conidia were observed in any of the cultures.

It was thought that possibly a rapid and abundant mycelial growth might be conducive to conidial production, so an attempt was made to find a medium upon which these species of Cercospora would grow readily. With this definite purpose, 30 kinds of media, which contained 40 different substances in varying quantities, were tried. It was convenient to classify these 30 kinds of media under four main groups: first, those containing cooked potato extract as the basic nutrient, plus additional salts and nutrients; second, those containing largely inorganic salts; third, host decoctions made by grinding the young succulent leaves of the respective host plants; and, fourth, those consisting wholly of sterilized host tissue.

The cultural characteristics of the 4 species of Cercospora used in the preliminary experiments, *C. beticola, C. davisii, C. dubia,* and *C. zebrina,* on the 4 main groups of media were as follows: In the first group, the growth was slow, aerial mycelium dirty gray, submerged mycelium olive green with numerous chlamydospore-like bodies; the aerial mycelium produced a gnarled, leathery mat usually forming oil droplets on the surface. In the second group, the growth was also slow, aerial mycelium gray, forming a gnarled, piled-up mat on the surface of the media with few chlamydospore-like bodies; oil droplets were produced over the surface of the mycelial mat. In the third group, the mycelial growth was dirty gray and much more rapid and abundant than in the other two, spreading over the entire surface of the media in about a week; very little submerged mycelium, and few chlamydospore-like bodies were produced. The nutrients in this group consisted largely of host decoction to which were added small amounts of dextrose and in some cases inorganic salts. Slowest growth was made on media in the fourth group; small amounts of light gray mycelium were produced, while in many instances there was cessation of growth in about 3 weeks.

It can be stated in general that those 4 species grew slowly, producing little mycelia on any of the media except numbers 14, 15 and 16. These media consisted of inorganic salts, juice from the beet plant and reduced agar base containing 1.2 per cent agar agar. Although these 4 species of Cercospora grew well on the media bearing the above numbers, no conidia were observed in any of the cultures at the end of a period of 2 to 3 weeks.

In view of the fact that no conidia were observed in the preliminary studies, new isolations were made of *C. beticola, C. davisii, C. dubia, C. physalidis* and *C. setariae,* in the fall of 1931 at Ames, and Kanawha, Iowa. D. H. Latham of Duke University very kindly supplied a culture of *C. cruenta* Sacc.

In the summer of 1932 4 additional species of Cercospora were collected and isolated, namely: *C. althaeina* Sacc., *C. avicularis* (Wint.) Hew., *C. muhlenbergiae* Atk., and *C. medicaginis* Ell. collected at Kanawha, Iowa.

Cercospora mirabilis Sharp and *C. moricola* Cooke were isolated from collections made by D. V. Layton at Conesville, Iowa.

Conidial Production from Newly Made Conidial Isolations[3]

Isolations were made from diseased leaves that had been placed in a moist chamber. After the leaves had remained in this environment from 15 to 20 hours, abundant production of conidia had formed in the necrotic spots. With the aid of a binocular microscope and a moistened, sterilized needle, there was little difficulty in isolating pure cultures of these fungi on potato-dextrose-agar slants. If a moistened, sterilized needle is brought just sufficiently near to the apical end of the conidia, the moisture on the tip of the needle will cause them to dislodge and adhere to the needle tip. These conidia, when transferred to agar slants, give a high percentage of pure cultures.

Certain species of Cercospora, as *C. dubia,* were difficult to isolate in pure culture, unless the above method was used. It appears that the conidia of this species were not so easily dislodged at the basal end as in the other species considered. However, if a leaf producing conidia remains in the moist chamber for about 15 to 20 hours, the conidia become easily dislodged and may be readily isolated in pure culture.

Within 36 to 72 hours after isolation small sparse mycelial colonies were produced on the surface of the nutrient-agar slants. Microscopic examinations showed (Table 1) that abundant conidial production had taken place in the following cultures: *Cercospora beticola, C. dubia, C. cruenta, C. davisii, C. physalidis,* and *C. setariae.*

Transfers were made from these isolations to potato-dextrose and sugar-beet-leaf-agar slants, and continued to sporulate when transferred at regular intervals to fresh agar slants. The method used in making these conidial transfers consists first of plunging a heated transfer needle into a sterile nutrient agar slant; then, by lightly scraping the moist, cooled needle over the surface of the culture, abundant conidia are caused to adhere to it. These conidia were transferred to the fresh nutrient-agar slant by lightly scraping the needle bearing the conidia over the entire surface of the medium to insure the development of a large colony.

The greatest number of conidia are produced by the conidiophores developed from the young mycelia arising from conidia. As the developing mycelia progressively covered the surface of the medium, fewer and fewer conidia were produced. There was greater vegetative growth and less sporulation in the case of *Cercospora beticola* on potato-dextrose agar, while the same organism on beet-leaf agar produced little vegetative

[3] By "newly-made isolations" is meant the isolation of conidia from diseased specimens collected from the living plant or from a recently collected specimen.

TABLE 1.—*The sporulation of 6 species of Cercospora in pure culture*

Cercospora species	Isolations		Pathogenicity	Cultural methods in maintaining sporulations			Interval of sporulation after transfer		Extent of sporulation
	Date	Host		Period of sporulation in months	Number of generations	Transfer intervals in days	Starting in hours	Stopping in days	
C. beticola	10/22/31	*Beta vulgaris*	+	3	20	3	40	8–12	Abundant
C. cruenta	1/10/32	*Vigna sinensis*	–	1.4	7	4	48	10–14	"
C. davisii	10/22/31	*Melilotus alba*	+	3	20	4	48	10–15	"
C. dubia	10/22/31	*Chenopodium album*	+	2	12	6	72	10–15	Numerous
C. physalidis	10/22/31	*Physalis* sp.	–	2	12	5	72	10–12	"
C. setariae	10/22/31	*Setaria glauca*	+	3	20	3	36	10–12	Abundant

growth and greater numbers of conidia for the first 4 or 5 days. However, the fungus seemed to grow freely thereafter, vegetatively, until the tenth day; then the growth was slow and was soon checked by dessication of the medium.

The amount of conidial production is affected by the kind of nutrient substratum, although the failure to culture these fungi on a specific medium did not prove to be the limiting factor in conidial production. *Cercospora beticola* fruited most abundantly when cultured on sugar-beet-leaf agar.[4] *C. cruenta, C. davisii, C. dubia, C. physalidis,* and *C. setariae* fruited abundantly on ordinary potato-dextrose agar.

When the sugar-beet-leaf medium was tubed and sterilized for 15 minutes at 12 pounds' pressure, it retained its leaf-green color. If the sterilization process is greatly prolonged, or at higher pressure, the medium becomes discolored and apparently is less suited to the growth of *Cercospora beticola* as shown by decreased conidial production.

A reduction in the amount of agar agar from the standard formula of 1.5 per cent to that of 1.2 per cent per liter solution was more conductive to conidial production. By using 1.2 per cent agar agar a medium is produced that is sufficiently firm to maintain the slants, but produces a comparatively soft substratum with more free water throughout, as well as on the surface of the nutrient substratum. These fungi grow more rapidly and sporulate more abundantly under such conditions.

As reported by Coons and Larmer (2), heavy mycelial growth was not followed by conidial production or especially conducive to it. This fact was verified in this laboratory; although in some cases a few conidia were produced on cultures where the mycelial growth was abundant, the most desirable type of medium for culturing these fungi was one on which very little aerial mycelium was produced during the first 5 or 6 days after conidial transfer. As shown in table 1, the minimum time for conidial production after conidial transfer varied with the species of Cercospora. The minimum time was 36 hours, the maximum, 72 hours, in case of *C. setariae* and *C. dubia,* respectively.

A medium suited to a particular species for optimum conidial production may not prove satisfactory in the case of other species of Cercospora. This is shown by *C. beticola,* which grew rapidly and fruited most abundantly on sugar-beet-leaf agar, while a 1.2 per cent potato-dextrose agar

[4] The sugar-beet-leaf agar used was a modification of "Coon's cheap synthetic medium" and consisted of 300 grams of freshly picked, young sugar-beet leaves, and 12 grams of agar agar per liter of solution. The sugar-beet leaves were finely ground to express the cell sap. This was then added to 1000 cc. of distilled water that contained 1.2 per cent melted agar agar and boiled 5 minutes, then strained through 2 thicknesses of cheesecloth, tubed, and autoclaved for 15 minutes at 12 pounds pressure.

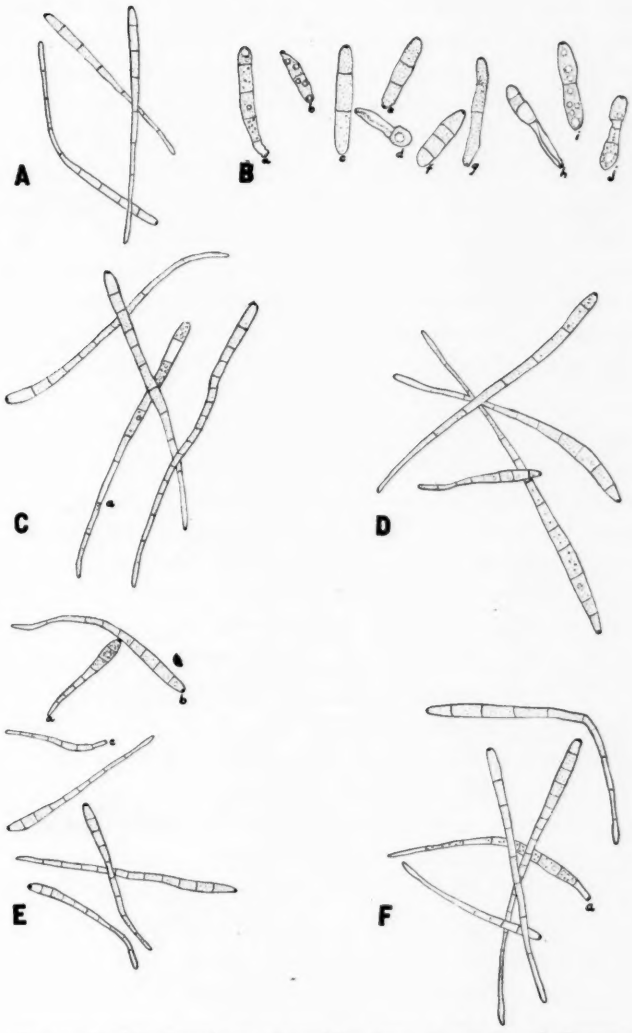

FIG. 1. Camera-lucida drawings of conidia from 6 species of Cercospora produced in pure culture. ×880. A. *C. physalidis.* Normal conidia from 5-day-old culture. B. *C. dubia. c, e, f,* and *g* are normal conidia from 5-day-old culture. *a, b, d, h, i,* and *j* from 2- to 3-week-old cultures. These partially collapsed or disintegrated conidia are commonly found in 2- to 3-week-old cultures of the species of Cercospora studied. C. *C. cruenta.* Normal conidia from 5-day-old culture, except *a,* which shows empty cells within a conidium from 2-week-old cultures. D. *C. davisii.* Normal conidia from 5-day-old culture. E. *C. setariae.* Normal conidia from 3-day-old culture. *a, b,* and *c* from 2- to 3-week-old cultures. Large oil globular formations are taking place, with *c* showing one empty cell in the process of disintegration. F. *C. beticola.* Normal conidia of 5-day-old cultures except *a,* which shows partially disintegrated conidia from 2-week-old culture.

produced abundant conidia in the case of *C. cruenta, C. davisii, C. dubia, C. physalidis* and *C. setariae.* It is of interest to note that *C. setariae* fruited equally well on potato-dextrose and on sugar-beet-leaf agar.

Normal conidia were produced in pure culture by 6 species of Cercospora, as is shown in figure 1. The conidia were all hyaline and exhibited a wide range of measurements. Conidia of *C. beticola* ranged from 65 to 450 μ, *C. cruenta* 30 to 250 μ, *C. davisii* 50 to 203 μ, *C. dubia* 25 to 102 μ, *C. physalidis* 55 to 210 μ, *C. setariae* 40 to 175 μ. Figure 1 also shows that after these cultures were 2 or 3 weeks old, few normal conidia were found. Certain of these conidia apparently "break down" or become disintegrated. In the early stage of this disintegration the smaller oil globules, present in the older conidia, coalesce to form a few large globules. This is followed by a shrinking of the cell content within an individual cell of a conidium. As this process continues, the cell or cells of a conidium will become completely devoid of their cell contents. Whether a small portion of the cell wall disintegrates where the germ tube normally would develop or at some other point is not known. It is also possible that the cell walls are partially dissolved away by the "staling products" thought to be produced under pure-culture conditions. This is merely supposition, but the fact that the condition stated does occur may explain why other investigators have failed to observe conidial production in cultures that were several weeks old. In other cases conidial germination in culture was also observed.

Hopkins (6) and Schmidt (14) reported the finding of conidia in old cultures. This might be explained by a rapid drying out or desiccation of the media, in which case a complete disintegration and germination of all the conidia possibly would be prevented. Abundant infection was obtained in cases where the respective host plants were inoculated with a conidial suspension of conidia grown in pure culture. Complete host-inoculation studies were omitted, since host plants were unavailable.

During the summer of 1932 at Kanawha, Iowa, 6 additional species of Cercospora were collected and isolated, namely: *C. althaeina, C. avicularis, C. muhlenbergiae, C. moricola, C. mirabilis,* and *C. medicaginis.* These fungi were grown on potato-dextrose agar with the exceptions of *C. mirabilis* and *C. moricola,* which were grown on sugar-beet-leaf agar. The same technique as used as that followed in culturing the preceding species. The cultures were held at a temperature of approximately 25° C. over a period of 2 months and during this time 12 consecutive conidial transfers were made at 5-day intervals. Throughout the entire period abundant conidia were produced.

MAINTAINING CULTURES IN A SPORULATING CONDITION

When it had been learned how to induce species of Cercospora to fruit from isolated conidia, the matter of maintaining these cultures in such a state became the consideration. In an attempt to determine the influence of interval of transfer on conidial production, a series of potato-dextrose agar and sugar-beet-leaf agar slants were planted with conidia, the latter being used for *C. beticola* only. These cultures were run in triplicate with 10 replications in each case. This series began with transfers from cultures 3 days old and continued with cultures 15 days old. Transfers made from 3- to 8-day-old cultures showed comparatively little difference in habit of growth and amount of sporulation. This may be explained by the fact that cultures at 8 days still retained an abundant supply of normal conidia. Although many conidia had germinated or disintegrated until only a few normal cells were present, these, when transferred to fresh slants, gave rise to new colonies. However, as the transfer intervals were lengthened there was macroscopic evidence that more vegetative growth was taking place than in cultures with shorter transfer intervals. Thus, by continuing this procedure, and by macroscopic as well as microscopic observations, the duration of conidial production (Table 1) was determined. During these 7 to 20 consecutive transfers sporulation was maintained, although at the end of this time there was some evidence of loss of vigor as compared with newly isolated cultures. In order to maintain some species of Cercospora in a sporulating condition it seems to be necessary to transfer spores rather than mycelium.

Subsequently, transfers were made from cultures varying in age from 1 to 5 months. In most cases there were a few conidia, but they were less abundant than in cultures that had been transferred at regular intervals of 5 to 6 days. The cultures also grew more slowly and produced many sterile hyphae. In general, the transfer of mycelia yields only sterile hyphae. Coons and Larmer (2) also observed the tendency of cultures to lose their power of fruiting when grown in artificial culture for long periods of time.

SUMMARY

The following 12 species of Cercospora were studied: *C. althaeina*, *C. avicularis*, *C. beticola*, *C. cruenta*, *C. davisii*, *C. dubia*, *C. muhlenbergiae*, *C. moricola*, *C. mirabilis*, *C. medicaginis*, *C. physalidis*, and *C. setariae*.

In preliminary experiments, using transfers from stock cultures of *C. beticola*, *C. dubia*, *C. davisii*, and *C. zebrina*, to 30 different kinds of media, no conidial production was observed.

Transfers from sporulating cultures yielded abundant spores, while transfers of mycelium gave largely sterile hyphae.

New isolations yielded abundant conidia in pure culture under favorable conditions in 36 to 72 hours.

Through the use of definite transfer intervals ranging from 4 to 6 days, conidia producing cultures were maintained from 5 weeks to 3 months.

Certain of these species of Cercospora demonstrated some specificity of medium, as determined by the amount of sporulation.

BOTANY AND PLANT PATHOLOGY SECTION,
AMES, IOWA.

LITERATURE CITED

1. ADERHOLD, R. *Mycosphaerella cerasella* n. spec., die Perithecienform von *Cercospora cerasella* Sacc., und ihre Entwicklung. Ber. Deut. Bot. Ges. **18**: 246–249. 1900.
2. COONS, G. H., and F. G. LARMER. The physiology and variations of *Cercospora beticola* in pure culture. Papers Mich. Acad. Sci. **11**: 75–104. 1929.
3. DUGGAR, B. M. Two destructive celery blights. A. Early blight. New York (Cornell) Agr. Exp. Sta. Bul. 132: 201–206. 1897.
4. ————. Three important fungous diseases of the sugar beet. New York (Cornell) Agr. Exp. Sta. Bul. 163. 1899.
5. GARMAN, P. The relation of certain greenhouse pests to the transmission of a geranium leaf spot. Maryland Agr. Exp. Sta. Bul. 239. 1920.
6. HOPKINS, E. F. Studies on the Cercospora leaf spot of bur clover. Phytopath. **11**: 311–318. 1921.
7. JENKINS, W. A. The cherry leaf spot fungus, *Mycosphaerella cerasella* Aderh., its morphology and life history. Phytopath. **20**: 329–337. 1930.
8. JONES, L. R., and C. S. POMEROY. The leaf blotch disease of the potato caused by *Cercospora concors*. Vermont Agr. Exp. Sta. Ann. Rept. **19**: 236–257. 1907.
9. KLOTZ, L. J. A study of the early blight fungus, *Cercospora apii* Fres. Michigan Agr. Exp. Sta. Tech. Bul. 63. 1923.
10. MCKAY, M. B., and V. W. POOL. Field studies of *Cercospora beticola*. Phytopath. **8**: 119–136. 1918.
11. NAGEL, C. M. The sporulation and host range of six species of Cercospora. Unpublished thesis. Library, Iowa State College, Ames, Iowa. 1932.
12. ————, and S. M. DIETZ. Sporulation of five species of Cercospora in pure culture. (Abs.) Phytopath. **22**: 20. 1931.
13. RAND, F. V. Some diseases of pecans. Jour. Agr. Res. **1**: 303–338. 1914.
14. SCHMIDT, E. W. Untersuchungen über die Cercospora-Blattfleckenkrankheit der Zuckerrübe (Aus der Abteilung für Pflanzenkrankheiten der Zuckerfabrik Klein-Wanzleben vorm. Rabbethge und Giesecke A. G.). Ztschr. Wissen. Biol. Abt. F. **1**: 100–137. 1928.
15. SINGH, U. B. Studies in the genus Cercospora. Jour. Ind. Bot. Soc. **10**: 73–91. 1931.
16. SOLHEIM, W. G. Morphological studies of the genus Cercospora. Ill. Biol. Monog. **12**: 1–85. 1930.
17. STOLZE, KARL VIKTOR. Beitrag zur Biologie, Epidemiologie und Bekämfung der Blattfleckenkrankheit der Zuckerrübe (*Cercospora beticola* Sacc.). Arb. Biol. Reichsans. Land-u. Forstw. Berlin-Dahlem. **19**: 337–402. 1931.
18. WELLES, C. G. Taxonomic studies of the genus Cercospora in the Philippine Islands. Amer. Jour. Bot. **12**: 195–218. 1925.
19. ————. Cercospora leaf spot of eggplant. Phytopath. **12**: 61–65. 1922.
20. WOLF, F. A. Pomegranate blotch. Jour. Agr. Res. **35**: 465–469. 1927.

APPLICATION OF STREAM DOUBLE REFRACTION IN THE IDENTIFICATION OF STREAK DISEASES OF TOMATO[1]

WILLIAM N. TAKAHASHI AND T. E. RAWLINS

(Accepted for publication November 20, 1933)

Several workers have demonstrated that necrosis of tissues in stems, leaves, and fruits of the tomato plant may be caused by infection with different viruses or combinations of viruses. Among the viroses showing this symptom are those commonly called the "streak" diseases.

We have been concerned with two of these diseases; one, which is well known and that several workers have called "combination streak," is caused by the potato latent virus when combined with tobacco-mosaic virus; the other, for which Shapovalov (2) proposed the name "dieback streak," is caused by a virus, or combination of viruses, which has not been identified. The combination streak was first described by Johnson (1) and the dieback streak by Shapovalov (2). As is evident from the descriptions given by these workers, the symptoms of the 2 diseases are somewhat similar and in certain cases it is difficult to distinguish them.

We have recently studied the stream double refraction exhibited by juice from tomato plants manifesting these diseases with the hope that this technic might be applicable in differentiating them.

Some of the specimens of dieback streak were taken from plants grown in a greenhouse at Riverside, California, and artificially inoculated by M. Shapovalov with this streak (all "B" specimens in table 1); others were naturally infected plants collected by Shapovalov in southern California (all "Q" specimens in table 1) and by M. W. Gardner and O. C. Whipple in central California (all "G" specimens in table 1). The combination streak used was always produced in the greenhouse at Berkeley by inoculating plants with a mixture of the potato latent virus and tobacco-mosaic virus.

Since previous experiments (4) have indicated that the method of juice extraction has a marked influence on stream double refraction 3 methods of extraction were used throughout these experiments. In the tables MC indicates that the tomato tops were macerated in a mortar and the juice was pressed out and centrifuged; MCFC indicates that juice obtained as above was frozen overnight at 0° F. and then thawed and recentrifuged; MFC indicates that the macerated tissue was frozen overnight, thawed, pressed

[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

TABLE 1.—*Summary of stream double-refraction experiments in the identification of streak diseases of tomato*

Disease	Plant number	Date	Critical dilution			Number of Nicotiana glutinosa leaves inoculated	Average number of lesions per leaf	Locality where plants were collected
			MC	MCFC	MFC			
Combination streak	1	5–25–33	1–256	.....	1–341	.....	.....	Greenhouse (inoculated)
" "	2	"	1–256	.....	1–256	.....	.....	" "
" "	3	"	1–341	1–512	1–512	.....	.....	" "
" "	4	"	1–256	1–341	1–512	.....	.....	" "
" "	5	"	1–512	1–768	1–1024	.....	.....	" "
" "	6	8–19–33	1–512	1–512	1–768	10	535.9	" "
" "	7	"	1–256	1–256	1–341	10	399.0	" "
" "	8	"	1–512	1–512	1–768	10	399.2	" "
Tobacco mosaic	1	8–19–33	1–128	1–196	1–196	10	186.4	" "
" "	2	"	1–256	1–341	1–512	10	85.3	" "
" "	3	"	1–512	1–512	1–768	10	182.5	" "
Dieback streak	B–1a	.....	1–16	.....	Slight	.....	.....	Southern California
" "	B–2a	.....	1–16	.....	"	.....	.....	" "
" "	B–3	.....	1–16	.....	"	.....	.....	" "
" "	B–4	.....	1–128	1–6	1–8	.....	.....	" "
" "	B–5	.....	1–16	1–4	1–4	.....	.....	" "
" "	B–6	5–29–33	1–47	1–4	Slight	.....	.....	" "
" "	B–7	"	1–16	1–2	0	.....	.....	" "
" "	B–1b	"	1–32	1–3	1–2	.....	.....	" "
" "	B–2b	"	1–24	1–2	Slight	.....	.....	" "
" "	Q1	8–3–33	1–4	0	Slight	10	0	" "
" "	Q2	"	1–16	Slight	1–4	10	0	" "
" "	Q3	"	1–16	"	1–6	10	0	" "
" "	Q4	8–9–33	1–8	"	Slight	5	0	" "
" "	Q5	"	1–8	0	1–2	5	0	" "
" "	Q6	"	1–8	0	1–3	5	0	" "
" "	Q7	"	1–6	0	Slight	5	0	" "
" "	Q8	"	1–12	1–2	1–3	5	0	" "
" "	Q9	"	1–12	0	0	5	0	" "
" "	Q10	"	1–8	0	0	5	0	" "
" "	Q12	"	1–16	1–3	1–6	5	0	" "

TABLE 1—(*Continued*)

Disease	Plant number	Date	Critical dilution			Number of Nicotiana glutinosa leaves inoculated	Average number of lesions per leaf	Locality where plants were collected
			MC	MCFC	MFC			
Dieback streak	Q13	8–9–33	1–6	Slight	Slight	5	0	Southern California
" "	Q14	"	1–8	"	1–6	5	0	" "
" "	Q15	"	1–8	1–4	Slight	5	0	" "
" "	G1	6–1–33	1–8	Slight	Slight	.....	.....	Clayton
" "	G2	"	1–12	1–3	1–3	.....	.....	Concord
" "	G5	6–6–33	1–12	1–4	1–4	10	0	Merced
" "	G6	6–5–33	1–6	Slight	Slight	10	0	Concord
" "	G7	6–17–33	1–16	1–6	1–4	10	0	"
" "	G8	"	1–12	1–4	1–4	10	0	"
" "	G9	6–16–33	1–12	1–4	1–4	10	0	San Jose
" "	G10	6–14–33	1–16	1–8	1–8	10	0	Concord
" "	G12	6–16–33	1–12	1–8	1–8	10	0	San Jose
Normal plant	1	5–25–33	1–32	1–6	Slight	.....	.....	Greenhouse
" "	2	"	1–6	1–2	0	.....	.....	"
" "	3	"	1–24	1–6	Slight	.....	.....	"
" "	4	"	1–32	1–3	0	.....	.....	"
" "	5	"	1–32	1–4	0	.....	.....	"
" "	6	"	1–47	1–4	0	.....	.....	"

and the juice centrifuged. The stream double-refraction technic described in an earlier paper (3) was used. In each test the juice was diluted with distilled water until stream double refraction was no longer detectable. This dilution we have called the critical dilution and have indicated it as 1–16 etc. in the tables. "Slight" and "0" indicate that even the non-diluted juice showed very slight or no double refraction, respectively.

The juice prepared by maceration of tissues, followed by freezing overnight, pressing, and centrifuging, (MFC) was diluted with an equal volume of water and was brushed on the leaves of *Nicotiana glutinosa* plants in order to determine whether tobacco-mosaic virus was present in the plants under test. The average number of local lesions produced per leaf is shown in table 1.

As is shown in table 1, juice from plants having dieback streak exhibited stream double refraction indistinguishable from that shown by juice from healthy plants. In this disease the critical dilution was always low and under MCFC and MFC was lower than under MC. Inasmuch as the juice from these plants produced no local lesions on *Nicotiana glutinosa,* the absence of tobacco-mosaic virus was proved. (It is possible that the virus or viruses causing dieback streak might have produced symptoms on *N. glutinosa* had the leaves been inoculated immediately after the juice was extracted.)

Plants having combination streak regularly exhibited a very high critical dilution, which was usually higher under MCFC and MFC than under MC. The stream double refraction exhibited by combination streak was indistinguishable from that shown by plants infected with tobacco-mosaic virus alone.

The above results demonstrate that the stream double-refraction technic may be used to distinguish plants having dieback streak from those infected with combination streak.

SUMMARY

Juice from streaked tomato plants infected with a combination of tobacco-mosaic and potato latent viruses exhibits a stream double refraction indistinguishable from that exhibited by tomato plants infected with tobacco-mosaic virus alone.

Juice from tomato plants infected with dieback streak exhibits a stream double-refraction behavior, which is indistinguishable from that shown by normal plants. This technic may therefore be used to distinguish diseased plants infected with combination streak from those infected with dieback streak.

LITERATURE CITED

1. JOHNSON, JAMES. Transmission of viruses from apparently healthy potatoes. Wis. Agr. Expt. Sta. Res. Bull. 63. 1925.
2. SHAPOVALOV, M. The dieback form of tomato streak. Phytopath. 23: 928. 1933.
3. TAKAHASHI, WILLIAM N., and T. E. RAWLINS. Rod-shaped particles in tobacco mosaic virus demonstrated by stream double refraction. Science n.s. 77: 26–27. 1933.
4. ————. Stream double refraction exhibited by juice from both healthy and mosaic tobacco plants. Science n.s. 77: 284. 1933.

PHYTOPATHOLOGICAL NOTES

Cytological Changes in the Callus of the Graft Union in Connection with Curly Top in Tomatoes.—It was reported by Shapovalov[1] that when an approach graft is made to unite a healthy tomato plant with a virus-affected one, as in the case of the curly-top infection, transmission of the virus occurs in a majority of cases. However, there is always a small percentage of grafts in which virus transmission does not result. A microscopic study of the tissues in the graft region reveals peculiar changes in the cell structure.

Callus tissues, including the graft union, were killed in Němec killing fluid, sectioned 5 μ thick, stained with acid fuchsin, and counterstained with light green. In these sections the surface of the union was made apparent,

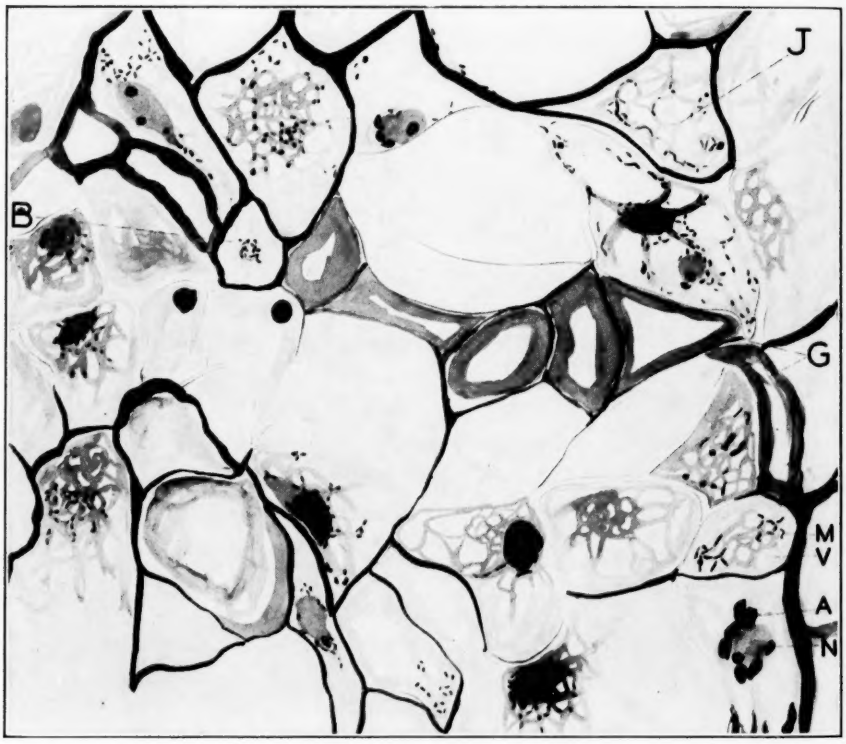

FIG. 1. Cellular structure through a graft union of tomatoes infected with curly top. B. Bridging over of proliferated cells. J. Cells that have reverted to meristematic condition. G. Degenerated cells at the surface of contact. M. Mitochondria. V. Vacuoles. A. Amyloplasts. N. Nucleus.

[1] Shapovalov, M. Graft transmission of curly top in tomatoes (tomato yellows). Phytopath. **21**: 998–999. 1931.

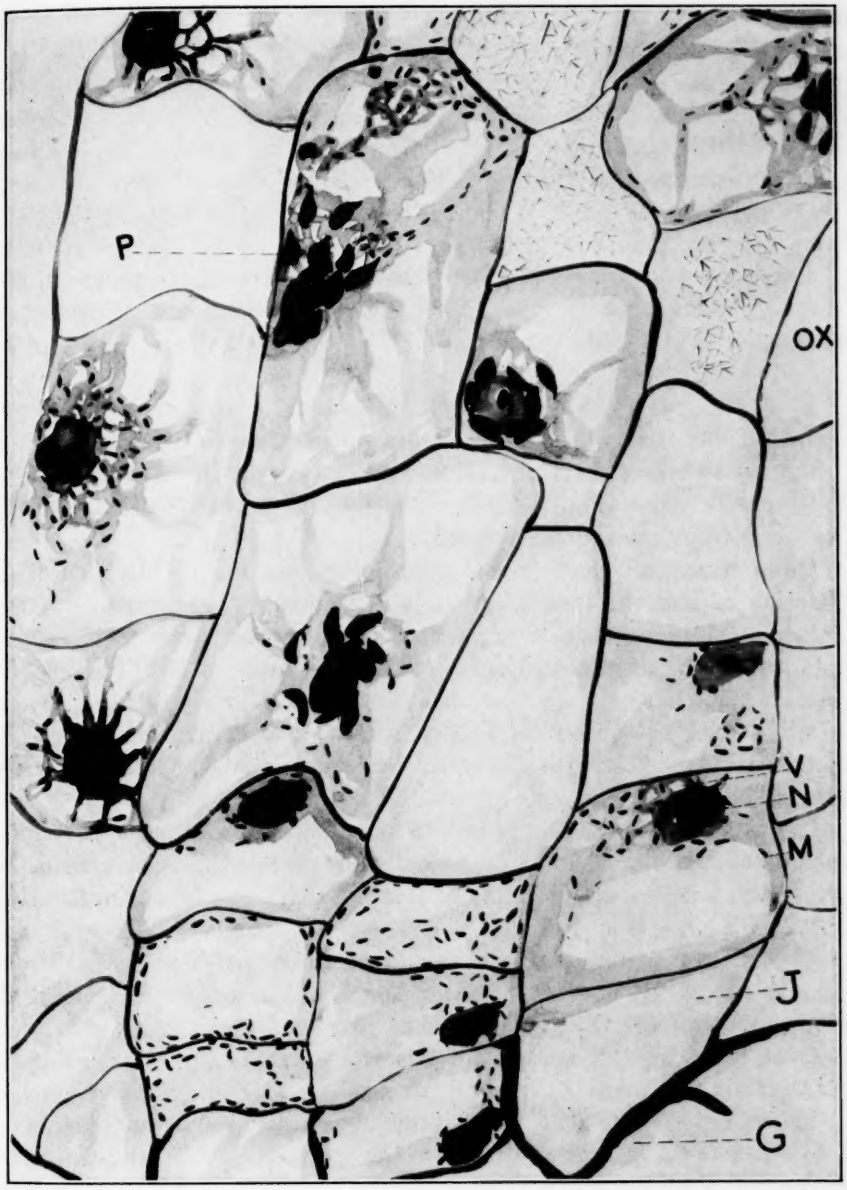

FIG. 2. Formation of calcium oxalate crystals near the contact surfaces of the graft union in a curly-top-infected tomato. OX. Crystals of calcium oxalate. P. Cells showing polarization, with amyloplastids grouped around the nucleus and numerous mitochondria grouped at the farther end of the cell. V. Vacuoles. N. Nucleus. M. Mitochondria. J. Meristematic condition of the cells, up and left. G. Degenerated cells at the graft union.

through united calluses, by a row of cells showing "gummy" (probably pectic) degeneration (Figs. 1 and 2, G). On each side of this row, cells reverted to the meristematic condition (Figs. 1 and 2, J). They contain no plastids, but only mitochondria (Figs. 1 and 2, M), and their cytoplasm is honeycombed with numerous small vacuoles (Figs. 1 and 2, V). Through a hyperplastic proliferation of these cells living tissues bridge across the row of degenerated cells, thus bringing about a contact between the living cells of the two plants (Fig. 1, B).

It may be readily seen, therefore, that after grafting, the virus can pass from one plant to another, although this movement is not so readily accomplished as in the tissues of the same plant, because of a barrier of degenerated cells.

Kostoff,[2] in grafting one species of Nicotiana to another, obtained precipitation phenomena in the expressed sap of the grafted plants, which he postulated to result from the formation of precipitins, as a response to grafting. In terms of antibody formation, he discussed his results as being of general significance in immunology.

Quite recently, Whitaker and Chester[3] gave a simple biochemical explanation of Kostoff's precipitation phenomenon. They pointed out that species of Nicotiana may differ as to the relative concentration of calcium and oxalic acid ions in their tissues. When the juices of 2 different species are mixed, as after grafting, calcium oxalate may be precipitated. Chemical tests in the laboratory proved that calcium oxalate is involved in this precipitation but no microchemical observation was made to localize the calcium oxalate in the tissues, *in vivo*.

It, therefore, seems of interest to note in this connection that large amounts of calcium oxalate were actually precipitated in some cells of the callus, a few layers away from the contact surfaces of grafted tomatoes (Fig. 2, OX).

Thus, the following principal changes were observed in the callus tissues formed at the graft union between an apparently healthy and a curly-top-infected tomato: (1) Death and a gummy degeneration of many of the cells adjacent to the contact surfaces of 2 grafted plants; (2) reversion to the meristematic condition of the underlying cells, and a bridging-over process by hyperplastic cells; and (3) a very abundant production of calcium oxalate in some cells, deeply situated in the callus.—J. DUFRÉNOY, Directeur Station de Pathologie Végétale du Sud-Ouest, Bordeaux, France, and MICHAEL SHAPOVALOV, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

[2] Kostoff, Dontcho. Acquired Immunity in Plants. Genetics **14**: 37–77. 1929.

[3] Whitaker, T. W. and K. S. Chester. Studies on the precipitin reactions in plants. Amer. Jour. Bot. **20**: 297–308. 1933.

A Fungus Gall on Viburnum Mistaken for Crown Gall.—Several years ago galls on *Viburnum opulus* L., the European cranberry bush, were received from an entomologist of New York State. He could find no insect, nor traces of any, to which responsibility for the trouble could be attached and thought it might be a case of crown gall. The shrubs affected were in a public park; the galls were spreading to other plants of the same species and some of the original infected ones had dead or dying branches. A few to many galls occurred on the stems and were ¼ in. to 1 in. or more in diameter (Fig. 1). They occurred at and just above and below the nodes and usually encircled the stem. The outgrowths consisted of root primordia; and, as this condition may occur in crown gall, work was undertaken to establish the presence of *Bacterium tumefaciens.* That organism could not be isolated from either of two lots of material. A fungus, however, was found present in several galls of both lots. This was isolated and inoculated into young *Viburnum opulus* plants the following spring, while the plants were growing well. No infection came from inoculations with the fungus, so the cause had to be sought elsewhere. Requests were made to the superintendent of the park where the disease occurred to send specimens at various times in the summer when the galls were small or just starting to form. This he did, and on the young galls that arrived in midsummer tiny flies and eggs of mites were found. The former were identified as *Cecidomyia* sp., and at the time were thought to be responsible for the galls.

An interval of 10 years elapsed, during which the writer received galls on *Viburnum opulus* at various times. These were considered to be Cecidomyia galls. In the summer of 1933, however, the specimens she received in various stages of development were studied by staff members of the Bureau of Entomology who could find no insect responsible for the galls. So, in search of a pathogen, isolations were made from the 1933 material and the same fungus that was isolated in 1923 was obtained. Inoculations by means of needle pricks were made into *Viburnum opulus* and *V. tomentosum* Thunb. in the spring and summer of 1934. This time galls were produced on *V. tomentosum* in less than 2 months, but up to the present time, July 20, none has developed on *V. opulus,* the native host. The galls produced are of the nodular type of the original galls. The stems punctured with a sterile needle for controls showed no outgrowths.

As the fungus enters through a wound, it seems probable that a mite or aphis may be associated with the parasite in the production of galls.

The fungus responsible for the disease is a Phomopsis. It is being studied to learn its relationship to the Phomopsis species known to be pathogenic.—NELLIE A. BROWN, Bureau of Plant Industry, Washington, D. C.

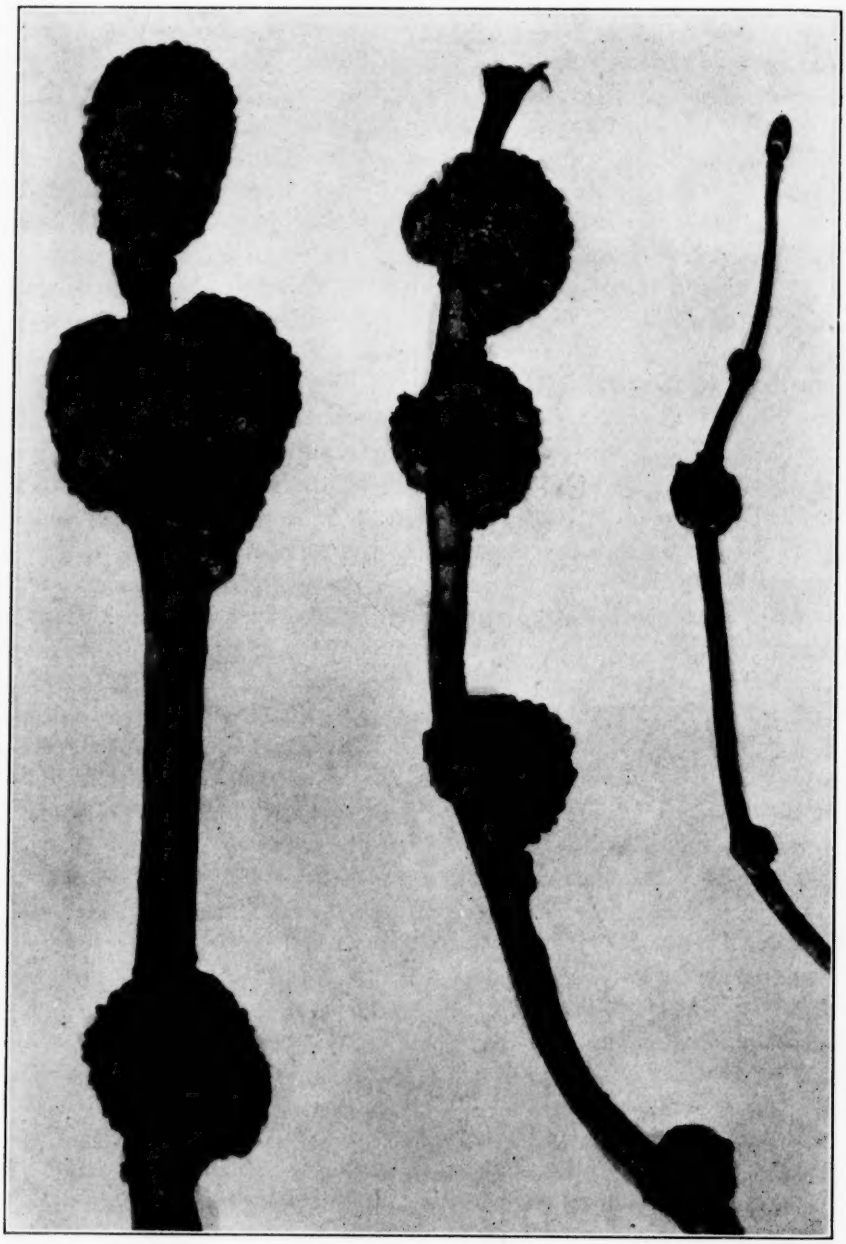

FIG. 1. Galls on *Viburnum opulus* from which Phomopsis was isolated.

White Pycnia and Aecia of Puccinia Graminis.[1]—In the routine testing of various species, varieties, and hybrids of Berberis for susceptibility to stem rust (*Puccinia graminis* Pers.), seedlings of *B. vulgaris* L. were inoculated in successive series with teliospores of *P. graminis agrostidis* Eriks. that had been stored at 0–2° C. for more than a year. Light to moderate infection resulted. Among the usual yellow pycnia there appeared a number of white pycnia. The pycniospores from both were hyaline, but the nectar of the white pycnia was colorless instead of the normal yellow. The difference in color, therefore, was in the nectar rather than in the spores.

Nectar was transferred from the white pycnia to white and to yellow pycnia. No aecia developed under the white pycnia treated with white nectar, but yellow aecia formed under some of the white pycnia treated with yellow nectar. Seedlings of *Agrostis alba* L. and of Manchuria barley were inoculated with these aeciospores, but no uredia developed.

A second set of inoculations was made on young barberry seedlings with teliospores of the same collection and a number of white pycnia again appeared. These infected barberries were placed under bell jars as soon as the infection became evident and were kept covered to exclude possible contamination. Ten days later nectar was transferred from white pycnia to white pycnia, from white pycnia to yellow pycnia, and from yellow pycnia to white pycnia. In a few cases aecia developed under the white pycnia treated with nectar from other white pycnia. These aecia were white, while those that developed under yellow pycnia treated with white nectar were yellow.

The number of "crosses" attempted is small, but the results indicate that white pycnia treated with white nectar form aecia about as readily as the normal yellow ones. Of the white × white crosses, 3 out of 7 resulted in the production of aecia. As a result of 23 crosses between white and yellow pycnia, 5 aecia appeared. No aecia formed under the one white pycnium treated with yellow nectar. As previously stated, the aeciospores formed under white pycnia to which white nectar had been transferred were white, while those formed under yellow pycnia to which white nectar had been transferred were yellow, as were the naturally formed aecia under the compound yellow pycnia. The length, size, rate of growth, etc., of the yellow aecia and the white aecia were similar under the conditions in the bell jars where the "crossing" was done and where the aecia formed.

[1] Cooperative investigation between the Minnesota Agricultural Experiment Station and the Project of Barberry Eradication, Division of Plant Disease Eradication, Bureau of Entomology and Plant Quarantine, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The investigation was undertaken before the Project of Barberry Eradication was separated from the Bureau of Plant Industry.

These white aecia were similar in size to and but slightly different in shape from the normal yellow aecia. The spores were hyaline and the intercalary cells in the chains were more conspicuous than those in the yellow chains. The aeciospores of both germinated normally in barley extract, and the germ tubes from the hyaline spores were hyaline.

A record was made of the numbers of white and yellow pycnia obtained from 2 sets of inoculations of successive series of *Berberis vulgaris* seedlings. In the first set there were, out of a total of 74, 21 white pycnia, constituting 28.38 per cent of the total number. In the second set of 102 there were 16 white pycnia, or 15.68 per cent. In the 2 sets the total number of pycnia was 139 yellow and 37 white, the white pycnia constituting 21.02 per cent of the total number in the 2 sets of inoculations.

The white pycnia were not distributed at random over the leaves of the infected barberries but were found more commonly on the leaves on one side of the plant. Also, the proportion of white to yellow pycnia on the same leaf was much higher on the one side of the barberry plant than on the other, and in a few cases white pycnia only were observed on leaves of the one side of the plant, while on the other side all were yellow. Thus, there were indications that the sporidia giving rise to white pycnia were produced on certain stems rather than at random throughout the collection of telial material, as the inoculations were made by suspending telial material above the barberry plants.—RALPH U. COTTER, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

Mercury Ammonium Silicate as a Gladiolus Corm Treatment.[1]—The effectiveness of calomel in the control of gladiolus scab has been proved.[2,3] Important commercial growers of gladiolus corms have reported its cost excessive when used at a concentration of 1 pound to 10 quarts of water, as recommended. In dipping corms in such a suspension it also was noted that the coverage of the corms was not uniform. In order to obtain a more even coverage of a toxic material, the possibilities of a mercury-ammonium silicate gel prepared by the action of a solution of mercuric bichloride on a mixture of ammonium hydroxide and sodium silicate were investigated.

Two formulae were employed in making the mercury silicate gels that differed only in solubility. The results were more satisfactory with the less soluble material. The coverage on the corms with this mercury gel was very uniform and complete, particularly when small amounts of a spreader were added.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Pathology.

[2] Tilford, P. E. Corm treatment for gladiolus and calla lily. (Abst.) Phytopath. **21**: 121. 1931.

[3] Miles, L. E. Control of gladiolus scab. Phytopath. **23**: 802–813. 1933.

Seven commercial varieties of gladiolus have been used in these tests with a total of 34 replications. Tests have been conducted also on gladiolus corms infected with *Penicillium gladioli.*

In all cases the mercury-ammonium silicate dip was compared with the standard calomel dip and a 7-hour soak in mercuric bichloride 1–1000.

TABLE 1.—*Summary of experiments in control of gladiolus scab*

Treatment	Clean corms	Corms with			Mummies
		1–4 lesions per corm	5–10 lesions per corm	over 10 lesions per corm	
		1932			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Check	23.4	39.8	22.3	12.7	1.8
$HgCl_2$	49.7	36.5	7.3	2.6	3.9
Calomel	75.5	21.0	0.7	.....	2.8
M. A. S.[a]	83.8	14.1	.....	0.2	1.9
M. A. S. ½ Hg. conc.	61.9	30.8	3.5	.....	3.8
M. A. S. ¼ " "	52.4	31.4	9.6	4.8	1.8
		1933			
Check	17.3	42.6	19.9	13.0	7.2
$HgCl_2$	57.2	38.0	4.5	.....	0.3
Calomel	87.4	12.0	.....	.....	0.6
M. A. S.[a]	90.7	9.3	.....	.....	.....
M. A. S. ½ Hg. conc.	83.1	16.4	0.4	.....	0.1
M. A. S. ¼ " "	69.1	30.2	0.6	0.1	.....

[a] Mercury ammonium silicate with mercury concentration equal to standard calomel dip.

From an examination of the data presented in the table, it is evident that the less soluble compound used in 1933 gave better protection against scab infection than the more soluble compound used in 1932. In both cases, where the mercury silicate was used at mercury concentrations equal to the standard calomel dip, control was essentially the same. When used at one-half the mercury concentration of calomel, the effectiveness was markedly reduced in 1932 but only slightly so in 1933, when the less soluble gel was used.

The experiments with *Penicillium gladioli* in 1931, 1932, and 1933 were conducted on Gen. de Wett, a white-flesh, susceptible variety. Calomel in 1931 increased the percentage clean corms from 17.2 in the check to 22.0 in the dipped lot. Mercury-ammonium silicate in 1932 increased the percentage clean corms from 18.9 in the check to 49.7, while in 1933, the percentage clean corms was increased from 43.4 per cent in the check to 69.7

per cent in the treated lot.—RICHARD P. WHITE, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

The Effect of Mosaic on Bloom Production of the Talisman Rose.[1]—Observations in commercial rose houses indicated that mosaic infection on the Talisman variety did not markedly interfere with growth or bloom production. This was so strikingly unlike the reaction of the suscept Madame Butterfly, which had been observed to produce less than 25 per cent of normal blooms, that the matter was thought worthy of investigating.

In April, 1932, the writer was called upon to rogue a large shipment of potted Talisman plants received from Oregon as dormant buds. When potted and grown for benching, they showed very vigorous growth. By the time they were ready for the bench, however, several had developed very marked and characteristic symptoms of mosaic. Thirty-six such plants were selected for planting in a small experimental greenhouse and 36 plants of the same lot, but showing no symptoms after a most careful examination, were selected for comparison purposes.

During the first 3 months of growth in the bed, 8 of the plants selected as healthy developed mosaic symptoms. During the following 21 months no additional mosaic plants were noted. This indicates that, at the time of benching, the most thorough examination of the growing plants is not adequate to effect a complete elimination of infected plants.

These plants, 28 healthy and 44 mosaic-infected, have been grown for 2 successive years in ground beds. The number of blooms cut, the length of stem from the base of the bud to the cut so made as to leave 2 leaves on the remaining stub, and the number of imperfect blooms have been recorded. During the second year of their growth notes also were taken on foliage symptoms of mosaic, since foliage showing severe symptoms would render that cut unsalable. A certain percentage of cuts showing slight symptoms on the foliage could probably be marketed.

A summation of the data for 2 production years showed that mosaic-free Talisman plants, under the conditions of this experiment, produced annually a total of 31.98 blooms per plant, with an average stem length of 10.77 inches. The mosaic-infected plants produced 34.78 blooms per plant with an average stem length of 10.55 inches. The increased number of blooms per plant on the latter is due to a tendency of mosaic-infected plants to lose the normal dominance of the terminal bud.

The percentage of imperfect blooms cut from the healthy and diseased plants was 2.90 and 7.55, respectively. During the second year, the cuts from the mosaic-infected plants showed 10.7 per cent with severe foliage

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Pathology.

symptoms and 7.5 per cent with slight foliage symptoms. The former were considered unsalable.

Assuming that the same percentage of cuts during the first year showed foliage symptoms rendering them unsalable, the production for the 2 years can be expressed on a basis of salable blooms per plant per year. On this basis the mosaic-free plants produced annually 31.05 blooms per plant and the mosaic-infected plants produced 29.07 blooms per plant.

These data indicate the relatively slight influence from a practical viewpoint that mosaic-infection has on the Talisman variety.—R. P. WHITE, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

Increase of Tobacco-Mosaic Virus in the Absence of Chlorophyll and Light.—In normal green leaves of certain varieties of the pepper, *Capsicum frutescens* L., infection with tobacco-mosaic virus is accompanied by production of necrotic local lesions. In variegated leaves of the same plants, white and apparently chlorophyll-free areas respond to inoculation by the production of similar lesions. Since the appearance of such lesions is regularly accompanied by and presumably dependent on virus increase, it seems to be indicated that multiplication of tobacco-mosaic virus is not dependent upon presence of chlorophyll.

This conclusion is supported by the results of a hitherto unpublished study of rate of increase of tobacco-mosaic virus in Green Mountain potato stems. Tubers were kept in pots of moist soil in a windowless cellar room, guarded by a double door against light from outdoors. A screen consisting of several layers of black cloth was placed around the pots to prevent accidental exposure to artificial illumination. When etiolated stems, with rudimentary leaves, had reached a length of a foot or more, pieces of stem 2 to 3 inches long were cut, inoculated with tobacco-mosaic virus by means of pin punctures, and inserted in previously sterilized test tubes. It is believed that these stems were free of chlorophyll. The work of cutting and inoculating the stems was carried out within a period of less than 20 minutes, with light as dim as seemed feasible for accurate handling. The inoculated stems in their tubes were then shielded, as before, by layers of black cloth in the dark cellar room. They were not again exposed, even to dim light, until they were to be tested. From time to time samples of the stems were crushed and their undiluted juices were used to inoculate plants of *Nicotiana glutinosa* L. Increasing numbers of lesions on *N. glutinosa* plants in successive tests furnished evidence of virus increase. The average number of necrotic lesions from 2 tests on the day of stem inoculation was 0; 1 day after inoculation, 1; 2 days after inoculation, 1; 3 days, 12; 5 days, 58; 7 days, 193; 10 days, 503; 12 days, 408; 14 days, 437; and 16 days, 641. These measurements gave evidence that virus did increase, al-

though in this experiment neither chlorophyll nor light was present. The rate of virus increase was comparable to that occurring in green leaves and stems of Green Mountain potato in the course of systemic necrosis induced by inoculating tobacco-mosaic virus into green plants under ordinary conditions of lighting.

In another experiment, using similar methods, a normal or nearly normal rate of virus increase was found in whole tomato plants placed in darkness 1 day before inoculation, and tested subsequently at intervals of 1 to several days. In this experiment chlorophyll was present but light was excluded.

There may remain some doubt as to the adequacy of the experimental conditions for total exclusion of chlorophyll and light. The evidence furnished by these observations leads to the conclusion, however, that tobacco-mosaic virus is able to increase normally under experimental conditions that would ordinarily be considered to exclude chlorophyll, light, and, consequently, the process of photosynthesis.—FRANCIS O. HOLMES, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

Aphid Transmission of Potato Yellow Dwarf.—The natural means by which the disease known as yellow dwarf of potatoes is transmitted under field conditions has not yet been determined. The high percentage of yellow dwarf in many commercial seed stocks suggests that some active insect vector is concerned. The virus has previously been transmitted mechanically and is regularly perpetuated through seed tubers. While the disease is recognized chiefly by the yellowing and dwarfing of the leaves, a rusty discoloration of the pith of the stem and tubers is a valuable diagnostic symptom.

Experiments[1] were undertaken in order to test various sucking insects as possible vectors under greenhouse conditions. The usual methods of technique were used. Twenty-five to 50 aphids were transferred from yellow dwarf Irish Cobbler potatoes to each of the plants of the varieties Bliss Triumph, Rural New Yorker, or Irish Cobbler under test.

The inoculated plants together with suitable controls were maintained for observation in a warm greenhouse for at least 6 weeks. The stems were then slit longitudinally and examined for rusty discoloration, which is believed to be a satisfactory diagnostic symptom for current infection.

As shown in the following table, a fairly high average percentage (60.5 per cent) of infection with yellow dwarf was secured with the peach aphid, *Myzus persicae* Sulz., on the 3 varieties of potatoes. The results with the potato aphid, *Macrosiphum solanifolii* Ashm., and with the onion thrips,

[1] Supported by the University of Wisconsin Research Fund.

TABLE 1.—*Transmission of Yellow Dwarf by the Aphid, Myzus persicae Sulz*

Variety inoculated	Number of plants tested	Number of plants infected	Number of non-inoculated controls	Number of controls infected
Rural New Yorker	70	57	53	3
Irish Cobbler	55	28	27	0
Bliss Triumph	55	24	50	0

Thrips tabaci Lind., have been mostly negative in the trials conducted thus far. It appears quite probable, therefore, that, under field conditions, the peach aphid may be the active vector of potato yellow dwarf.—KARL KOCH, University of Wisconsin, Madison, Wis.

Medium for Growth of Pythiaceous Fungi.[1]—The maltose malt medium described by Leonian, in his study of factors promoting pycnidium formation in some Sphaeropsidales[2] has been used successfully as a medium for the growth of Phytophthora species.

After boiling the ingredients together a short time, a clear amber-color extract may be obtained by filtration. It remains clear during subsequent autoclaving, and also with sufficient phosphoric acid or potassium hydroxide to give reactions from pH 1.5 to 11.5, added aseptically after autoclaving. It is very useful in studies requiring the weighing of mycelium, as well as in detailed microscopic studies.

A modification of Leonian's maltose malt medium, substituting dextrose for maltose, and using easily weighed quantities of the ingredients, to expedite preparation, has given as good growth as has his medium. The modified formula is as follows: KH_2PO_4—1.0 gram, $MgSO_4$—0.5 gram, Bactopeptone (Difco standardized)—1.0 gram, Difco extract of malt, desiccated powder—5.0 grams, dextrose—15.0 grams, water—1 liter. Autoclave at 15 pounds for 30 minutes. For the solid medium, add 15 grams of agar-agar (Difco standardized) per liter.

This medium has been used for 3 years in this laboratory for all stock cultures of Pythiaceous fungi and for saprophytic and parasitic imperfect fungi. These include all species of Phytophthora, Nematosporangium, and Pythium studied by Sideris[3] for which he recommended media prepared

[1] Published with the approval of the Director as Miscellaneous Paper No. 8 of the Pineapple Experiment Station, University of Hawaii, Honolulu, Hawaii.

[2] Leonian, L. H. A study of factors promoting pycnidium-formation in some Sphaeropsidales. Amer. Jour. Bot. **11**: 19–50. 1924.

[3] Sideris, C. P. Taxonomic studies in the family Pythiaceae. I. Nematosporangium. Mycologia **23**: 252–295. 1931.

from honey dew melon seed, watermelon seed, or papaya fruit. Vegetative growth and reproduction on it are superior to those on corn-meal agar and Leonian's succinic acid medium[4] and equal to that on oat or papaya agars. Phytophthora species[5] grown on it readily produce zoospores when transferred to dilute media.[6]

Either Leonian's maltose malt medium or the modification of it, given above, has been more satisfactory than other media ordinarily used for culturing the Pythiaceae, from the standpoint of ease of preparation, ability to precisely duplicate composition, and availability, both as a clear liquid and a clear solid of comparable composition.—F. P. MEHRLICH, Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii, Honolulu, Hawaii.

[4] Leonian, L. H. Differential growth of Phytophthoras under the action of malachite green. Amer. Jour. Bot. **17**: 671–677. (p. 673). 1930.

[5] While growth of *Phytophthora infestans* occurs on this medium, it has not been compared with growth on other media.

[6] Mehrlich, F. P. Taxonomy and host range and asexual reproduction of Phytophthora species causing heart rot of pineapple plants. Phytopath. **24**: In press. 1934.

Paul Sorauer, *Handbuch der Pflanzenkrankheiten.* Fifth edition. Volume III. Die pflanzlichen Parasiten. Pp. VIII + 948. Paul Parey, Berlin, 1932. Bound, 66 Reichsmarks.

The second volume of Sorauer's manual, which dealt with plant diseases due to bacteria, to slime molds, to phycomycetes, and to ascomycetes, made its appearance in 1928. In the present volume, one of the largest to be issued in this or in any previous edition of the set, are treated the diseases of living plants and the damage to plant products attributable to destructive forms among the basidiomycetes, the fungi imperfecti, the algae, the lichens, and the seed plants. The unexpectedly long lapse of four years between the dates of publication of the two parts concerned with parasites and epiphytes belonging to the plant kingdom is explained in a preface by Appel, one of the editors, as being due mainly to an increasing realization of the need for a complete revision of the earlier edition. Moreover, the deaths of Noack and Hosterman made necessary the appointment of four new collaborators to work up the important section devoted to the fungi imperfecti.

The present volume contains no section corresponding to the chapter on the control and prevention of plant diseases due to fungi that took up 34 of the 310 pages in the corresponding volume of the fourth edition; the matter covered therein evidently being reserved for expanded treatment in a separate volume dealing with plant protection announced forthcoming by the publishers as the sixth and concluding unit in the set. Yet, in spite of this diminished scope, the book under review contains more than three times as many pages as its predecessor of 1923. The more extensive treatment has effected an immense improvement in the utility of every section. The expansion of the section on the rusts from 59 to 132 pages has enabled Köhler to give due attention to the important discoveries on the biology of these parasites made during the last two decades, in large part in the United States and Canada. The section on the Ustilaginales consisting of 147 pages contributed by Zillig, sets forth our knowledge of the smuts with a completeness not approximated in his earlier account of 39 pages. Laubert's treatment of the Exobasidiineae, in 15 pages, is incomparably more instructive than Lindau's earlier 2-page summary. Likewise, the section of 107 pages on the Hymenomycetineae, written by Münch, deals with the pathology of living trees and the biology of timber decay with a fullness impossible in the 17 pages of text contributed earlier by Lindau, much of which necessarily had to be devoted to taxonomic discussion. The 87 pages of the chapter on the Sphaeropsidales by Laubert and Richter have not only

permitted these authors a much fuller textual treatment than was afforded by the 22 pages in the corresponding section of the previous edition, but enabled them to include 31 figures in place of a single figure. Pape's account of diseases due to members of the Melanconiales occupies 83 pages and contains 16 illustrations, in comparison with 25 pages and 3 illustrations devoted to the analogous section of earlier date.

Nowhere has the expanded treatment resulted in more evident improvement than in the difficult section on the Hyphomycetes contributed by Wollenweber. Here, 253 pages, in which the uniformly admirable text is illustrated with 18 figures, replace 57 pages containing 3 figures. Much of the increase is apportioned to several genera whose status in plant pathology has been made much better known in recent times; among which may be cited Botrytis, Alternaria, Cercospora, Graphium, Verticillium and Helminthosporium. The lion's share of the increase, however, went to the chapter on Fusarium, to which is devoted a total of 87 pages, or 65 pages more than in the previous edition—an expansion to be accounted for in part, no doubt, by the predilection of the author. That this chapter constitutes virtually a monograph within the more general compendium will make the volume no less attractive to the numerous workers dealing with members of the genus to whom the same author's more extended account may be inaccessible.

A section of 13 pages on parasitic algae deals with a field of phytopathological interest too often ignored in American textbooks. Perhaps the citation here of several substantial papers on Cephaleuros and Rhodochytrium by American investigators may direct increased attention to the occurrence of algal plant parasites in the United States. A chapter of 10 pages from the pen of Pape disposes of the curious damage to plants due to lichens. The final chapter in the book, written by Köhler, and consisting of 32 well-illustrated pages, deals competently with the more familiar, yet far from commonplace, injuries attributable to parasitic seed plants.

The volume is concluded with an index occupying 50 pages of 3 columns each. Host plants as well as parasites and injurious epiphytes are listed both by their genera and by their common names in German. Diseases are listed mostly by their German designations, but often also by English terms when these have been widely used in the literature. In addition, numerous items are included having reference to discussions on the pathological, morphological, physiological, or biological aspects of particular diseases or of categories of diseases affecting either particular host plants or groups of host plants. References to the literature are given, as in previous editions, in footnotes and in such quantity that if they were assembled they would, in spite of the small type employed for them, fill more than 100 full pages. In thoroughness and documentation the present volume far outstrips not

only the corresponding part of any previous edition but also the companion volume of the same edition; or, for that matter, any compendium whatever covering the same ground. I anticipate there will be little occasion for such expressions of disappointment as were vented by some reviewers of Volume II, when they found omitted all mention of what naturally looms highest to investigators generally—their own individual contributions.

In view of the difficulty of avoiding a catalogue-like style in compositions dealing with innumerable items, the several authors have shown more than ordinary resourcefulness in making the text coherent and readable. The book seems in an unusual degree free from errors in statement, though minor departures from accuracy occur here and there. On page 677, for example, it is stated that knowledge concerning the very inclusive host range of *Helminthosporium giganteum* came to light through *"weiteren Beobachtungen und erfolgreichen Infektionsversuchen"* of mine. In the papers cited in connection with this statement, however, only infections that had come about spontaneously in the field were considered; and certainly no infection experiments of any kind were attempted with the fungus.

The more extended treatment has permitted the inclusion of many illustrations from comparatively recent publications to supplement the older figures hallowed by tradition. The half-tones among these newly introduced illustrations seem of exceptional merit. Some of the zinc cuts, however, lead one to suspect that the hand of the transcribing draughtsman was heavier than it was cunning. Perhaps that hand might have been employed to better effect in wielding scissors and paste-brush since nearly all of the figures in question were coarse enough as published originally to have permitted the necessary reduction in size to be accomplished by direct photographic methods. The depressing character of some other illustrations is hardly to be traced to maladroit transcription, but rather to their failure in the first place to represent properly the morphological features of the fungi they purport to represent. Of the figures assembled on page 835, only two are drawn well enough so that the direction of rotation in the individual sporiferous hyphae can be distinguished. In these two figures both sinistrorse and dextrorse conditions are shown, though the legends indicate that a dextrorse species was concerned in each case. It is undoubtedly in large part through illustrations of such quality that the lingering faith in the transitional position of Actinomyces between the bacteria and the fungi, which is reaffirmed in the unhappy sentence opening the special chapter devoted to that genus, is sustained.—CHARLES DRECHSLER, United States Department of Agriculture, Washington, D. C.

The Helminthosporium Disease of Oats. By the Department of Plant Husbandry [G. D. O'Brien and R. W. G. Dennis]. The West of Scotland

Agricultural College Research Bulletin No. 3. Pp. 74. With 23 plates and 8 diagrams. Glasgow, 1933.

A rather distinctive feature of the publications that have been issued now for a decade or more from our larger experiment stations in series designated as "Research Bulletins," is that they have permitted extensive discussion of special agricultural problems with regard more particularly to their purely scientific aspects. The present publication, though from a foreign source, is broadly similar to this class of domestic contributions not only in series designation, but in nature of content and in treatment, as well. An introductory section dealing with the "leaf-stripe" disease of oats in Scotland, and a historical review of more general scope, is followed by sections considering in successive order, etiology, growth of the causal fungus in pure culture, morphology of the fungus, duration of viability of its conidia, influence of environmental factors, pathogenicity of the fungus, leaf infection and pathological anatomy, biology of the fungus, control of the disease, and varietal susceptibility. The text is supplemented with appropriate graphs, workmanlike line-drawings, and excellent half-tone reproductions of photographs; and that in numbers so unstinted as to lead one to suspect that the country in which the memoir was published may be unjustly celebrated as a land of excessive frugality. A summary covering one page, and a bibliography citing 70 titles concludes this valuable contribution.—CHARLES DRECHSLER, United States Department of Agriculture, Washington, D. C.

The Genus Diaporthe Nitschke and Its Segregates. By Lewis E. Wehmeyer. University of Michigan Studies, Scientific Series, Vol. IX. 11 × 8 inches. Pp. XII + 349. With 18 plates. University of Michigan Press, Ann Arbor, Mich. 1933. Price, $3.50.

In this book the author, who has published extensively on Pyrenomycetes for more than a decade, undertakes the immense task of bringing order into the welter of more than 650 species that have accumulated in the genus Diaporthe since it was erected by Nitschke 67 years ago. Four genera, Diaporthopsis, Apioporthe, Diaporthella and Cryptodiaporthe, are recognized in addition to the basic genus, of which an amended definition is supplied. To this redefined genus, cited as Diaporthe Nit. Emend., are referred 70 different species not counting the recently described cranberry parasite, *Diaporthe vaccinii,* which is stated in an addendum to be closely related to *D. phaseolorum.* The latter species is definitely made to include as varieties two parasites of economic importance that have enjoyed specific rank under the binomials *D. sojae* and *D. batatis.* The pathogenic form known under the binomial *D.* (*Phomopsis*) *citri* likewise is shorn of its independent status, being listed as a variety of *D. medusaea.*

The segregated genera show a smaller, yet still respectable, membership, 19 approved species being listed under Cryptodiaporthe, 3 under Diaporthella, 8 under Apioporthe, and 6 under Diaporthopsis. Two species of Diaporthopsis are cited as not having been examined, and 1 receives attention in the chapter on "Doubtful species" together with 18 species of Diaporthe. Excluded from Diaporthe are 89 species, among them the very familiar *Endothia parasitica,* and also the rose-canker parasite, *Diaporthe umbrina,* which is held to be referable to Cryptosporella. A final chapter entitled "Species not seen" gives 149 names, all under the genus Diaporthe, together with the original descriptions in Latin, English, German, French, Spanish, or Magyar attaching to them.

The morphological criteria are accorded primary importance in distinguishing the various species from one another. Host relationships are, however, kept clearly in view by giving separate treatment to plurivorous forms under each genus or other convenient group of host plants on which they occur. Such separate treatment includes citations of synonyms, individual host species, countries from which the host relationship has been reported, pertinent exsiccati, herbaria or individual collections in which material representative of the host relationship is found, and conidial connections together with appropriate discussion of the fungus in relation to the particular host plant or group of host plants in question. The semi-diagrammatic drawings illustrating outward habits, though hardly elegant, are yet workmanlike and consistent in execution. Especially to be commended are the figures of the spores that appear at a uniform magnification of $\times 1000$—a uniformity certainly very desirable in any comparative work. The usefulness of the volume is enhanced by a well conceived index and by adequate keys to the species of the several genera treated. In a period of enfeebled finances it may not be inappropriate to add that the price of the book appears unusually reasonable.—CHARLES DRECHSLER, United States Department of Agriculture, Washington, D. C.

REPORT OF THE EIGHTEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The eighteenth annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of California, Berkeley, June 18–19, 1934, in conjunction with the fourth national summer meetings of the American Association for the Advancement of Science.

The attendance was very satisfactory, there being an average of 50 to 60 visitors at each of the 4 half-day sessions. Over 70 people, most of whom are affiliated with the Pacific Division, signed the register during the meetings.

At a business meeting the following officers were elected:

President.....C. O. SMITH, University of California Citrus Experiment Station, Riverside, Calif.

Vice President.....C. W. BENNETT, United States Department of Agriculture, Riverside, Calif.

Secretary-Treasurer.....B. A. RUDOLPH, University of California Deciduous Fruit Station, San Jose, Calif.

Councilor.....T. E. RAWLINS, University of California, Berkeley, Calif.

Of these officers, the President will serve one year, the others two.

A total of 44 papers were presented, the titles and abstracts of which follow the present report. The ever-increasing importance of viroses and the attention they are commanding from plant pathologists were exemplified in the large number of papers dealing with diseases of this type that were presented. The balance of the papers reported the results obtained in the study of fungus diseases and subjects pertaining to mycology, nematology, and bacteriology.

Many delightfully informal events punctuated the run of the meetings, not the least of which was the pathologists' dinner. The meal, typically Italian, was served in the evening at a well-known café in Oakland and served to bring the visiting members together under less formal auspices than those of the meetings. Also, many availed themselves of the opportunity to become acquainted with the work being done by the Division of Plant Pathology of the University of California, not only at headquarters in Berkeley, but also at the branch of the College of Agriculture at Davis and at the Deciduous Fruit Station at San Jose.

B. A. RUDOLPH, *Secretary-Treasurer*

Restoration Properties of Erodium cicutarium on the Attenuated Curly-top Virus.—C. F. LACKEY.

Testing Sugar Beets for Resistance to Curly-top.—N. J. GIDDINGS.

Properties of the Sugar-beet Curly-top Virus.—C. W. BENNETT.

Breeding for Resistance in Blackeye Cowpeas to Fusarium Wilt, Charcoal Rot, and Nematode Root Knot.—W. W. MACKIE.

The diseases, Fusarium wilt (*Fusarium tracheiphilum*), charcoal rot (*Rhizoctonia bataticola*), and nematode root knot (*Hederodera marioni*), seriously injure Blackeye cowpeas in the interior valleys of California, especially on sandy soils. Varietal tests in infested soil eliminated all varieties, except Iron, Victor, and Brabham, as breeding stocks. Of these, Brabham showed more damage from charcoal rot and nemas than the other two. Victor, while vigorous and resistant, yielded poor seed crops and shattered badly. Artificial crossing between Iron and Blackeye gave the best results. In the F_1 resistance to all 3 diseases was dominant, the seed was black and dappled brown and midway in size between the parents. In the F_2 color, both mottled and self, was dominant to the eye type. The eye type broke up into blackeye, browneye, and smokeye types, with blackeye dominant to the other two. There appeared to be no correlation between early maturity, size and color of seed, and resistance. Correlation appeared to exist between dark-colored leaves and disease resistance. Resistance to the 3 diseases lies in the root cortex, but quality rather than thickness determines resistance. It is suggested that suberin, known to exist in quantity in the Iron parent, an ancestor of Victor and Brabham, accounts for disease resistance. This point is receiving further study. Normal methods of segregation and back crossing to the Blackeye parent have both yielded satisfactory resistant blackeye types that lack only confirmatory tests before being placed in the infested areas.

A Method of Inducing Knots on Cuttings of the Oleaceae by Bacterium Savastanoi.—CLAYTON O. SMITH.

Typical knots were produced on cuttings of *Olea europeae, O. chrysophylla, Osmanthus aquifolium, O. fragrans* and *Forestiera acuminata* when these cuttings were inoculated through wounds with *Bacterium savastanoi*. The method followed was to select wood about a year old and make cuttings 6–12 inches long. The leaves were not removed except at the lower end, which was to go into the fine gravel of the rooting chamber. Three inoculations were made on the lower portion of the cutting, later inserted into the soil, and 2 placed on the upper part of the soil. Control cuttings were similarly treated but without inoculation.

In 4 to 6 weeks no roots had developed on cuttings, but overgrowth $\frac{1}{4}$–$\frac{1}{2}$ inch in diameter occurred at the point of injury. These were larger when they were in the soil. The control cuttings in nearly all cases showed no overgrowths. In a small amount of callus tissue formed, it was many times less than the overgrowth on the inoculated cuttings. Artificial inoculations on olives in the open made at the same time as on the cuttings developed typical knots. Inoculated cuttings when placed in gravel under a bell jar also developed knots.

Spotted Wilt of Head Lettuce.—C. M. TOMPKINS and M. W. GARDNER.

Heavy losses in the head-lettuce crop are caused by the spotted-wilt virus in certain coastal districts in California. Infected plants are unmarketable or die prematurely.

The disease causes a slight marginal wilting, necrotic spotting, and slight yellowing of the leaves, usually on one side of the plant, with consequent tilting of the head toward the affected side. Lateral curvature of many leaves is produced. Plants may become infected at any age. The necrotic spotting involves even the youngest leaves, and in older heads there may be difficulty in detecting the internal necrosis. Percentage of infection increases with age of crop.

All varieties observed, including Romaine, are susceptible. The virus has been transmitted by the rubbing method followed by an incubation period of 10 to 25 days. The virus from lettuce produced typical spotted wilt in tomato and that from spotted wilt in tomato produced typical symptoms in lettuce. Reciprocal inoculation has been successful between lettuce and a group including eggplant, Physalis, pepper, *Nicotiana rustica*, Salpiglossis, Schizanthus and aster. Local lesions have been produced on snapdragon and broad bean and systemic infection on lettuce by inoculation from calla lily, and lettuce has been infected from spotted wilt in nasturtiums.

Spotted Wilt of Tomatoes and Its Transmission by Thrips.—M. W. GARDNER and O. C. WHIPPLE.

Spotted wilt is a serious disease of tomatoes, especially in the cooler coastal regions, and of peppers and head lettuce. It is prevalent in nasturtiums and has been found also in aster, cineraria, Datura, petunia, dahlia, calceolaria and calla. The disease has been found in plant beds (tomato, pepper, eggplant, aster) in early spring. The virus has not been transmitted with the seed of tomato, pepper, or Datura. It has been transmitted by the rubbing method from tomato to tomato, Datura, potato, eggplant, pepper, Physalis, Salpiglossis, Schizanthus, Nicandra, *Nicotiana glutinosa, N. acuminata, N. rustica, N. sanderae, N. alata, N. langsdorffii*, tobacco, petunia, lupine, broadbean, dahlia, aster, zinnia, chrysanthemum, cineraria, lettuce, Martynia, nasturtium, and nettle, and has been recovered from Datura, pepper, *N. glutinosa*, tobacco, zinnia, lettuce and calla. Successful transmission by thrips has been obtained between tomato, pepper and Datura, from tomato to petunia, from Datura to aster, and from cineraria and calceolaria to tomato. In the warmer valleys the earlier necrotic symptoms are followed by stunting, mottling and leaf distortion, while in the cooler regions the necrosis may kill the plants (dieback or streak). Commercial control was obtained in a greenhouse by frequent fumigation and roguing. Infected plant beds are an important source of field infection.

A Destructive Virus Disease of Cauliflower and Other Crucifers.—C. M. TOMPKINS.

A serious and widespread virosis of cauliflower (*Brassica oleracea* var. *botrytis*) occurs in commercial plantings throughout the coastal areas of central California. Field losses vary from 20 to 30 per cent, exclusive of losses sustained during transit to eastern destinations. The field distribution of infected plants was general, without suggestion of localized spots. Severely diseased plants were stunted, while the dwarfed, terminal heads were surrounded by small, distorted leaves exhibiting prominent chlorosis, coarse mottling, and necrotic spotting. Chlorosis was generally confined to that part of the lamina between but not adjacent to the midrib or main lateral veins, with small islands of dark green tissue distributed throughout this area. Presumably where late infection had taken place, heads reached marketable size, but enroute to eastern markets the attached, diseased leaves turn yellow, materially reducing the value of the product. Essentially, a low temperature virus, it is readily transmissible by juice inoculations to leaves of healthy cauliflower seedlings. Under greenhouse conditions (55°–65° F.), the incubation period ranges from 12 to 20 days. Successful inoculations also have been

made to cabbage (*B. oleracea* var. *capitata*), and kale (*B. oleracea* var. *acephala*), annual stock (*Mathiola incana*) but not to any plants outside the family Cruciferae. This disease was found in commercial kale seed beds, and it is probable that seed beds may serve as the primary sources of infection of cauliflower seedlings. Roguing of diseased seedlings before transplanting offers a partial method of control.

Breaking in Stock (Mathiola incana), a Virosis.—C. M. TOMPKINS.

A systemic virosis of annual stock (*Mathiola incana*) occurs in commercial plantings and home gardens in the coastal areas of central California. The chief symptoms accompanying infection consist of slight to severe stunting of the plant and complete or sectorial breaking of the flowers in the terminal racemes, resulting in a bleached, blotched effect of the undersized petals, occasional mottling of the leaves, and reduction in size of the seed pods. All varieties, particularly American Beauty and Heatham Beauty, in the Giant Imperial and Giant Perfection groups of stock, single and double flowers, including the entire range of floral color from white to red, appear to be susceptible to natural infection. Broken flowers are valueless for the cut-flower trade and, where present, cause a loss. Young, healthy Imperial Crimson stock seedlings with 6 leaves were successfully infected by means of juice inoculations in the greenhouse at approximately 60° F. The incubation period varied from 5 to 7 weeks. Readings usually cannot be taken until the blooming period because of the inconstancy of leaf mottling. Sixty per cent of the inoculated plants became diseased, as proved by the broken flowers, while the non-inoculated controls remained healthy. The relation of this virus to the destructive cauliflower virus and its mode of spread in the field remain to be determined.

Growing Powdery Mildew on Detached Bean Leaflets and Breeding for Resistance.—BJARNE DUNDAS.

For use in inoculation tests, the powdery mildew (*Erysiphe polygoni*) of beans, *Phaseolus vulgaris*, was continuously propagated on detached bean leaflets supported on a 10 per cent sucrose solution in Petri dishes stored in the light at room temperature (19°–21° C.). Spores germinated well in dry or saturated air between 15° and 21° C. Field observations and dish tests showed the varieties Pinto, Hungarian, Yellow, and selections from Lady Washington and Pink to be resistant, while Robust, Small White, Red Kidney, Kotenashi, and many others were susceptible. Dish tests with detached leaflets showed that the Pink selection was susceptible when young and that it acquired resistance with age. Necrosis of invaded epidermal cells was associated with resistance. By using the dish test it was found that in the F_2 of hybrids of resistant and susceptible varieties, such as Pinto × Robust, a ratio of 3 resistant to 1 susceptible was obtained. This indicates that resistance to powdery mildew in the varieties tested is due to a simple dominant Mendelian factor.

Observations on the Basidial Stage of Sclerotium rolfsii.—J. T. BARRETT.

The basidial stage of *Sclerotium rolfsii* Sacc., apparently has not been reported from the United States. Cultures received in Formosa and Italy from this country have, however, been reported to produce that spore stage. Recently 2 cultures of *S. rolfsii* originating in the United States have produced the perfect stage, which has been compared with a culture of *Corticium rolfsii* (Sacc.) Curzi and with a basidium-producing strain of *Sclerotium rolfsii* Sacc. from New Zealand. Comparison of the vegetative and sclerotial characters were made with a culture of *Corticium centrifugum* (Sev.) Bres. received from Curzi of Italy.

The observations made to date suggest that the 2 cultures from the United States referred to and the New Zealand strain should, at least tentatively, be referred to *Corticium rolfsii* (Sacc.) Curzi.

A Chytridiaceous Parasite of Phytophthora.—J. T. BARRETT.

A parasite, a species of the genus Pleolpidium, was found in April on Phytophthora trapped from greenhouse soil in which snapdragon plants were suffering from a crown rot caused by *Phytophthora cactorum*. Of the few species of Pleolpidium described, none has been reported on Phytophthora. The parasite produces enlarged, usually spherical, swellings on the hyphae but, of more interest, it infects the sporangia. These cannot readily be distinguished from normal sporangia until the parasite has reached the swarm-spore stage. At the time of maturation of the swarm spores the finely granular character of the contents due to the small size of the swarm spores is seen in contrast to the much larger differentiated swarm spores of the host. Discharge of the small uniciliate swarm spores is through one or more very short hyaline exit tubes, which protrude through the wall of the host sporangium. The empty sporangium thus seen has the papilla of dehisence of the host sporangium left unbroken.

Successful inoculations of *Phytophthora cactorum* isolated from infected snapdragon plants with the parasite have been made.

A Bud Blight of Gooseberry Apparently Caused by Nematodes.—RALPH E. SMITH, H. N. HANSEN and C. EMLEN SCOTT.

A peculiar disease of English gooseberry has been under observation near Albion, Mendocino County, California, for a number of years. The disease is characterized by the nondevelopment of the leaf buds and spurs, or the production of a few deformed, stunted leaves from some of the buds. The lateral spurs remain very much shortened and somewhat swollen and club-like. The whole plant becomes stunted and its yield is greatly reduced.

No cause for this trouble was discovered until the present season, when a generous sample of diseased stems was gathered by a grower during a period of very foggy weather and brought immediately to the laboratory. Examination with the microscope then showed the presence of enormous numbers of nematodes in the diseased buds and also imbedded in the tissue of some of the partially developed leaves. The character, abundance, and location of these worms left little doubt as to their being the cause of the trouble.

No report of a disease of this sort has been found in America. In England, however, a similar disease is described on black currant, caused by *Aphelenchus ribis*. Dr. G. Steiner, Senior Nematologist, U.S.D.A., tentatively considers the Mendocino County material as *Aphelenchoides ribis*, which may prove to be a synonym of *A. fragariae*.

Quantitative Determinations of Sclerotium rolfsii in the Soils of Sugar-beet Fields.—L. D. LEACH.

Sclerotium rolfsii causes a serious root rot of sugar beets in California. A method of determining the approximate numbers of sclerotia in field soils has been devised. A composite of soil samples taken 8 inches deep from a definite area is dried, pulverized, screened, and passed through a Boerner sampler to obtain 4 400-gram aliquot parts. These are washed separately through a series of 3 screens (10-, 20-, and 40-mesh) under a strong stream of water and the sclerotia are recovered with forceps from a water suspension of the residue. Germination trials reveal the percentage of viability. In 1931 a 70 per cent infection on sugar beets produced a population of approximately

5,000 sclerotia per square foot of soil. Determinations in the same field in 3 successive years following barley plantings showed 1,100, 175, and 40 living sclerotia per square foot. In other fields the decrease following diseased beets has been less rapid. In one field a population of 150 living sclerotia per square foot produced a 20 per cent infection on sugar beets. This method is being used as a direct measure of the efficiency of crop rotations, cultural practices, fertilizers, and fungicides in reducing the infestation in experimental plots and in commercial fields.

Seed Transmission of Fusarium Yellows of Beans.—JAMES B. KENDRICK.

In 1929, Harter (Phytopath. 19: 84) briefly described a vascular Fusarium disease of field beans (*Phaseolus vulgaris*) observed in the Sacramento Valley, California. The disease was observed by the writer in this locality in 1929 and again in 1933. Bulk seed was harvested in 1933 by crushing diseased pink bean plants in a cloth bag and also by picking pods from diseased plants and opening them by hand so as not to contaminate the seed. This seed was grown in steam-sterilized soil in a greenhouse. Of the 482 plants grown from the bulk seed, 56, or 11.6 per cent, showed wilt, while 481 plants from the hand-picked seed showed no wilt. Seed from the crushed diseased plants was dusted with Semesan at the rate of 2, 4, and 8 ounces and Ceresan at the rate of 4 ounces per 100 pounds of beans and grown in steam-sterilized soil. One hundred and eighty-eight plants from the 2-ounce treatment showed 1 diseased plant; 117 plants from the 4-ounce treatment showed 2 diseased plants; 166 plants from the 8-ounce treatment, and 157 plants from the 4-ounce Ceresan treatment showed no disease. This evidence indicates that the spores of the causal Fusarium are carried on the seed coat and are deposited there during the harvesting operations.

Evidence Suggestive of the Existence of a Filterable Stage of Bacterium tumefaciens.—G. E. M. CLARK.

A single strain of *Bacterium tumefaciens* was grown in nutrient broth, and cultures of different ages (2 to 43 days) were passed through a Berkefeld "N" filter. The filtrates were inoculated into *Bryophyllum calycinum,* and also cultured in various ways. Out of 8 tests, 5 filtrates yielded *Bacterium tumefaciens* (pathogenic), though only one was directly pathogenic to Bryophyllum.

Similar tests made with milk cultures furnished no certain case of pathogenicity from direct inoculation, though 4 out of 6 filtrates contained *Bacterium tumefaciens,* which was pathogenic. Three tests made with filtrates from crushed crown galls produced only negative evidence.

Two originally identical strains, only one of which was pathogenic to Bryophyllum, gave rise to rough and smooth types of *Bacterium tumefaciens* after having been grown for 3 months in litmus milk. In the pathogenic strain, the rough organism was the more virulent.

Nonsterile Soil Leachate Stimulating to Zoosporangia Production by Phytophthora Sp.—F. P. MEHRLICH.

By growing *Phytophthora cinnamomi* or *Ph. parasitica* in a maltose malt-extract broth, followed by rinsing in sterile distilled water, and incubation in a nonsterile aqueous leachate of field soil, zoosporangia are produced more consistently and abundantly than by other published methods.

Autoclaving greatly limits the effectiveness of leachate, for the differences in the abundance of sporangia produced in sterile and nonsterile extracts are repeatedly of the order illustrated by Leonian (Amer. Jour. Bot. 12: 450. 1925) as concentrations II or

III and V, respectively. Neither aseptic aeration nor the addition of a trace of $Fe(NO_3)_2$ has increased the effectiveness of autoclaved leachate. Differences between pH of autoclaved and nonsterile extracts have not been correlated with numbers of sporangia produced.

Sterilization of soil, prior to leaching, using Pistor's method (reported to give bacteriologic sterility without markedly affecting solubility of ash, total acid or pH, MgO nitrogen, total nitrogen, or HCl hydrolizable nitrogen) has had the same limiting effect as autoclaving.

Sterile leachate has been superior to sterile M/100 KNO_3 or Petri's solution. Nevertheless, the use of Petri's solution inoculated with nonsterile leachate has given as good a production of sporangia as the original leachate. Thus it appears that factors associated with the presence of microorganisms of field soils are stimulating to copious sporangial production.

Using these methods, papillate zoosporangia of *Phytophthora cinnamomi* have been produced in considerable numbers contrasting with the blunt nonpapillate ones described by all other workers.

Laboratory Experiments on the Control of Brown Rot of Lemons.—L. J. KLOTZ, H. S. FAWCETT, and L. L. HUILLIER.

Bordeaux mixture, solutions of copper sulphate, and hot water (115°–120° F.) are the most effective means yet discovered for combating brown rot. Each, however, has one or more undesirable features. When followed within 2 years by hydrocyanic acid fumigation, a spray of Bordeaux mixture may cause severe injury to the trees. Copper sulphate in the wash water corrodes galvanized iron tanks and is precipitated if used in combination with soaps and alkaline washes. Hot water causes a liberation of rind oil and the resulting oil spottings when the fruit is cold and turgid.

A search is being made for effective materials not having these undesirable features, 89 different solutions of various chemicals having thus far been tried. The criteria for judging relative effectiveness were: (1) The period of motility of zoospores in a drop of watery suspension placed on a dried film of the material; (2) effect on germination; mounts prepared in (1) were placed in moist chamber and later examined for germination; (3) protection of fruit; to simulate orchard conditions, lemons sprayed and allowed to dry 20 hours were atomized with a zoospore suspension and subsequent infections noted.

The effectiveness of a chemical in wash water was estimated by the number of infections that developed after atomizing the lemons with a spore suspension, leaving undisturbed one hour and then immersing them for 2 minutes.

As a tree spray the most promising substitute yet found for Bordeaux is a mixture of zinc sulphate and hydrated lime, prepared by the usual procedure used in making Bordeaux. The relative efficacy of the 2 sprays is illustrated in one experiment in which 4–4–50 Bordeaux permitted an average of 1.7 infections per fruit, zinc sulphate-lime 3.4 infections, while the average per check lemon was 24.7. The present recommendation is to increase the zinc-lime content to make an 8–8–50 spray.

For use in wash water at packing houses, such mild alkaline washes as 1 to 2 per cent solutions of soda ash, trisodium phosphate, and sodium silicate have given highly promising results, approaching the effectiveness of the standard 1–8000 copper sulphate solution.

Some Factors Influencing the Character of Interaction Between Trichoderma and Other Soil Fungi.—R. WEINDLING.

The character of interaction on nutrient media between Trichoderma and other soil fungi is determined by various internal and external factors. By varying such en-

vironmental conditions as acidity, moisture, or temperature, the parasitic action of Trichoderma may be suppressed and the colonies may show either mutual inhibition or compatibility. When planted opposite Rhizoctonia in a Petri dish, Trichoderma sometimes will not coil around Rhizoctonia hyphae. Direct attack, however, can be induced by sowing Rhizoctonia on an agar suspension of Trichoderma spores. After a definite period following spore germination in the solidified agar, Trichoderma shows maximal parasitic activity.

These phenomena can be connected with the properties of a lethal principle of Trichoderma. Probably additional mechanisms are involved, such as staling effects, enzyme production, etc.

Different groups of Trichoderma are influenced differently by the above factors. The action of Trichoderma is affected also by the host, *e.g.*, in the case of Pythium, lethal effect is easily obtained but is rarely accompanied by coiling around the hyphae.

The interaction between members of any pair of microorganisms may thus be of a dynamic nature similar to that shown by mycorrhiza, or smuts on grains, *i.e.*, changes in conditions may transform compatibility between two organisms into an antagonistic or even parasitic relationship.

Various Fungi Recently Found to be Parasitic on Rhizoctonia solani.—R. WEINDLING.

Various fungi grown on culture media with *Rhizoctonia solani* attacked the latter in a manner resembling the parasitic action of Trichoderma. Under the conditions of these tests, their virulence diminished in the following order: *Acrostalagmus* spp., *Aspergillus niger*, *Penicillium* spp., *Fusarium lateritium*, and *Botrytis cinerea*, *Verticillium* spp. showed only a slight tendency to direct attack. Toxic substances produced by these molds seemed to be instrumental in killing and in preventing growth of Rhizoctonia when parasite and host were in actual contact as well as when they were at some distance from each other.

Thus it appears that many soil saprophytes are capable of this peculiar type of parasitic action. Various common molds are reported to be able to make use of dead microorganisms in their metabolism. Perhaps one of the reasons that they are so common in the soil lies in their ability to overcome their competitors in the struggle for food and eventually use the dead hyhae as nutrients.

The Use of Nitrogen Trichloride and Other Gases as Fungicides.—L. J. KLOTZ.

Nitrogen trichloride gas, in concentrations lethal to fungi of decay but not injurious to citrus fruits, is being employed with much promise to protect fruit in storage rooms and in loaded cars. Equivalent concentrations of chlorine, while more toxic to the fungi, injure the rind of fruit and thus prepare the way for greater losses. Ozone is but slightly inhibitory to the fungi and the rate of decay in inoculated oranges.

Concentrations of NCl_3 gas as low as 4 to 6 milligrams per cubic foot for a period of 30 minutes were lethal to heavy plantings of conidia of *Penicillium digitatum* and *P. italicum* on filter paper and agar. Mycelium of *Phytophthora citrophthora* and spores of *Colletotrichum gloeosporioides*, *Alternaria citri* and *Dothiorella gregaria* were similarly sensitive, but large clumps of mycelium were not penetrated and killed by these low concentrations of the gas. Microscopically, the protoplasts of gas-treated spores, germ tubes, and hyphae were seen to be shrunken and pulled away from the cell walls, and, in some cases, with marked granular and coagulation effects.

After 8 to 10 weeks' storage, losses in oranges due to decay may, as shown by numerous tests, be reduced 50 to 75 per cent or more by giving the fruit 3 to 5 three-hour treatments of 15 mg. NCl_3 per cubic foot at 3- or 4-day intervals, beginning the first day of storage.

A Survey Concerning a Native Pathogen, Armillaria Mellea.—J. LEE HEWITT.

In a highly developed citrus area and the watershed back of it, *Armillaria mellea* was found native and of wide distribution in infection centers that are locally isolated on hillsides and related to flood plains along stream beds. Hosts on hillsides are the oak, and along streams include the willow, poplar, and sycamore.

Distribution from natural habitat to groves is by flood water that deposits infected débris together with infectable débris among stream bank growth, which picks up and transmits the infection by its roots to near-by groves. Invasion of grove areas was more frequent and rapid formerly, in grapes, apricots, and walnuts than now, when citrus is predominant.

Oak, sycamore, and citrus seem resistant. Herbaceous plants were found infected.

Removal of stream-bed growth may retard dispersal.

Control efforts in citrus groves can take advantage of host characteristics and factors of fungus distribution. Treatment by carbon bisulphide or surgical removal may be used.

Experiments in Biological Control of Rhizoctonia Damping off.—R. WEINDLING and H. S. FAWCETT.

Experiments to protect citrus seedlings from Rhizoctonia damping off in sterilized soils by inoculating the latter with Trichoderma were more successful with some types of Trichoderma than with others and more so at acid than at neutral reactions. The interactions of the 2 fungi in sterilized soils paralleled those on culture media.

Attempts were then made to utilize this experience for biological control of the disease in nonsterilized soils. At moderately acid reactions soils inoculated with several Trichoderma types often gave considerable control, and the Rhizoctonia checks showed nearly total loss. In very acid soils almost complete control was obtained. The very acid Rhizoctonia checks showed, however, little and sometimes no damping off. Isolation from these Rhizoctonia checks proved that Trichoderma, which had not been purposely introduced, as well as some other fungi, increased in abundance with increasing acidity, while it became difficult or impossible to reisolate Rhizoctonia.

The growth of *Rhizoctonia solani* declines at very acid reactions. In sterilized soils, however, it attacks citrus seedlings in the whole range of reactions used above. The control of Rhizoctonia damping off through soil acidification may thus be due primarily to the increased parasitic, antagonistic, and competitive activities of other soil organisms, *e.g.*, Trichoderma.

Tobacco Mosaic on Spinach.—LEON K. JONES.

The common tobacco-mosaic virus (No. 1) has been used to inoculate Bloomsdale Savoy spinach. Mechanical juice inoculation has given from 50 to 90 per cent infection on spinach plants incubated 16 to 20 days. The first symptom of the disease is a mottling of the older foliage with slightly raised dark green areas in the lighter green tissue. As the affected plants age, increased puckering of the foliage with dwarfing of the plants is noted. The older mottled leaves become yellowed and may die a month to 6 weeks following inoculation.

Heterothallism in Corn Rust.—R. F. ALLEN.

Puccinia sorghi is a heteroecious, heterothallic rust alternating between corn and oxalis. The gametophytic, unisexual mycelium on oxalis produces spermogonia, stomatal hyphae, and small, rapidly deteriorating, haploid aecia. Only when the 2 sexes meet do fertile aecia develop. When spermatia of one sex are transferred to the surface

of an infection of opposite sex, a spermatium placed in contact with a paraphysis or a stomatal hypha becomes attached, its nucleus passes over into the hypha and moves in through it to the internal mycelium. Under favorable conditions a spermatium not in contact with any surface hypha can germinate and form a slender hypha that enters the nearest stoma and so becomes effective. Spermatial nuclei entering gametophytic hyphae divide rapidly and migrate through the hyphae. Twenty-four hours after spermatization 60 per cent of the vegetative cells throughout the mycelium contain more than one nucleus. The haploid aecia also are rapidly diploidized and then progress to spore formation. One fertilization suffices for a lifetime. All aecia forming after the fertilization will mature normally.

Heterothallism in Flax Rust.—R. F. ALLEN.

Melampsora lini (flax rust) is autoecious and heterothallic. The gametophytic, unisexual mycelium, characterized by cells containing 1 to several nuclei, produces spreading spermogonia with few, weak, irregular paraphyses. There are few or no stomatal hyphae, and doubtful aecial anlage. When spermatia of one sex are transferred to the surface of an infection of opposite sex they enter the leaf through epidermal cells or through spermogonia, grow for a brief time in the leaf tissue, and then pair with the native hyphae in the young aecia to form "two-legged" basal cells that give rise to chains of aeciospores.

The Parasitic Action of Omphalia sp. on Tissues of the Date Palm.—DONALD E. BLISS.

Lesions produced by *Omphalia* sp. constitute a specific, diagnostic symptom of decline disease, and they may be found in all underground portions of affected trees. When placed in soil about healthy plants, the fungus first becomes established in dead portions of the outer leaf bases. Rhizomorphic strands then penetrate to inner layers of leaves and grow along root surfaces where mats of mycelium become closely attached to the epidermis. Underlying cells are killed, their walls darken, and often collapse, before fungous invasion begins. In leaf tissue the starch grains disappear from cells at the margin of the advancing lesion. Apparently the fungus enters wound-free tissues, penetrating cell walls and filling all cavities, including the vascular elements, with closely woven masses of hyphae. Although small at first, lesions may enlarge gradually until entire sections of roots or leaf bases are killed. The abortion of young primary roots as they penetrate diseased leaf bases to reach the soil, constitutes the most damaging effect on the palm. Lesions 2 cm. in depth were observed in trunk tissue of old palms that were severely stunted and in that of young seedlings and immature offshoots. In the latter cases death resulted from fungous invasion of the terminal bud.

A Case of Almond Blossom Infection by Coryneum beijerinckii Furnishing Inoculum the Following Year.—E. E. WILSON.

Twig lesions are regarded as the most important means by which *Coryneum beijerinckii* passes from one season to the next. Examinations during the spring of 1934, however, showed the fungus sporulating on mummied blossoms that had been blighted the year before. It was further found that *C. beijerinckii* attacked the calyces during anthesis in 1934, and that in a few cases the lesions spread downward to the pedicel, blighting the blossom. Whether *C. beijerinckii* commonly blights almond blossoms remains to be proved. The great number of mummied blossoms in the orchards under observation may have first been attacked by *C. beijerinckii* and later blighted by the brown-rot fungus, which also was common.

Aside from the fact that *Coryneum beijerinckii* may pass from one season to the next, in certain cases, on dried-up blossom parts, is the equally important fact that calyx lesions constitute sources from which conidia may spread, after bloom, to the young fruit and leaves. It was observed that the calyces, even after abscission from the receptacle, adhered to the developing fruit and that numerous lesions subsequently developed on these fruits.

Localization of Symptoms During the Early Stages of Curly-top Infection in the Sugar Beet.—KATHERINE ESAU.

Phloem degeneration and the external symptoms of curly-top of the sugar beet become perceptible first in those parts of the plant nearest the inoculated leaf and directly connected with it by phloem tissue. This suggests that the virus is translocated in the phloem. Xylem seems not to be concerned in this movement, since phloem degeneration occurs in those traces where no xylem has yet differentiated.

The Rate of Spread of the Veinbanding Virus on Potatoes.—LEON K. JONES.

In the summer of 1933 plantings of from 2 to 13 hills each of 31 virus-free seedling strains and ¼ acre of virus-free Early Rose potatoes were made near Pullman, Washington, at least ½ mile from soil that had ever produced potatoes. The potato planting was surrounded by fields devoted to barley and alfalfa. The potato plants became naturally infected with the veinbanding virus during the growing season as follows: Thirteen seedling strains, 100 per cent; 6 seedling strains, over 50 per cent; Early Rose, 59 per cent; 5 seedling strains, 10 to 50 per cent; 7 seedling strains remained virus-free.

Types of Trichoderma Rot of Lemons and Oranges.—H. S. FAWCETT and R. WEINDLING.

The observations here recorded are based on inoculations made with 30 cultures of Trichoderma into wounds of mature lemons. The typical Trichoderma rot was described by Fawcett and Lee (*Citrus Diseases and Their Control*). It was always produced by cultures of *T. lignorum* having a coconut-like odor. This rot never was found with the nonodorous type of the species that is able to produce a yellow pigment. In a few cases this second type invaded the core of the fruit several weeks after inoculation, induced an internal rot, and finally broke out on the rind. A third type of Trichoderma, nonodorous and nonpigmented, caused a rot that differed from the typical one by showing a much lower percentage of infections, a firmer appearance, and very slow development. Using smaller numbers of cultures and fruits, the results obtained by inoculating mature Valencia oranges with these 3 types of *T. lignorum* were similar to those on lemons.

Some single-spore cultures from an odorous isolation of *Trichoderma lignorum* grew very slowly. They produced but little of the characteristic odor and showed no parasitism on lemons.

A culture of *T. Koningi* was found to cause a rather soft, easily punctured buffy brown rot.

Interspecific Anastomosis and the Origin of New Types in Imperfect Fungi.—H. N. HANSEN and RALPH E. SMITH.

Two species of *Botrytis* (*B. allii* and *B. ricini*) were observed to anastomose when grown together in co-cultures. Their progeny was analyzed through many culture series by single-spore methods. Of 20 single-spore isolations from the original mixed culture, 6 were identical with one parent, 9 resembled the other, while 5 were

decidedly different from either. By repeated single sporing from these aberrant cultures 3 types were segregated that appeared to be sufficiently distinct to be considered new varieties or even new species. These types have remained constant through 3 single-spore-culture series. Contemporaneous series from the original parents showed no variation. The suggestion is offered that the production of these aberrant forms from combined, interspecific cultures of imperfect fungi may result from genic changes induced by the presence of specifically different nuclei in the same cell brought together by the mechanism of anastomosis.

Dematophora Root Rot.—HAROLD E. THOMAS, H. N. HANSEN and H. EARL THOMAS.

This disease, first recognized as distinct from other root diseases about 5 years ago, is killing trees in a number of apple orchards in Santa Cruz County, California, and in at least one apricot orchard in Alameda County. The symptoms and associated fungus agree in essentials with published descriptions of the root rot caused by *Dematophora necatrix*. Pathogenicity of the fungus has been demonstrated with small apple and pear trees.

The disease may be distinguished from those caused by Armillaria and Xylaria by the absence of well-defined rhizomorphs or stromata and the presence of profuse, white, cottony mycelium adjacent to affected roots in moist air in the laboratory or in soil cavities in the orchard. Coremia of Dematophora are found occasionally near the ground line on trees killed by the disease. They usually are formed also in pure cultures of the fungus on steamed kernels of wheat and oats.

The Relation of Carbon-nitrogen Ratio and High Acidity to Color Production by Fusarium Species.—A. W. DIMOCK.

As the result of studies of the relation of the carbon-nitrogen ratio and the acidity of the substrate to the production of pigment by Fusarium species the following conclusions were adduced:

1. The factors affecting color production in the genus Fusarium differ with the species and even the strain involved.

2. The production of nondiffusible pigment by a gladiolus Fusarium was found dependent upon a very high acidity of the medium. The production of diffusible pigment by this organism was, however, conditioned by an actual deficiency in nitrates, the reaction of the substrate being only of indirect importance.

3. The production of diffusible pigment by *Fusarium lycopersici* var. *pisi* required a carbon-nitrogen ratio of greater than 1, but was not dependent on a deficiency in nitrates. The hydrogen-ion concentration seemed important only as it affected the growth of the organism.

4. It was seen that not only do the carbon-nitrogen requirements for pigmentation vary with different Fusarium species, but the nature of the required carbon source also varies.

Canker on Chamaecyparis lawsoniana.—J. W. HOTSON and D. E. STUNTZ.

Frequently, there has been brought to the attention of the nurserymen in the vicinity of Seattle a disease occurring on *Chamaecyparis lawsoniana,* involving the formation of a stem canker that eventually kills the tree by girdling. The cankers usually are produced on the stem just above ground level, or on the root collar, rarely higher on the trunk, apparently resulting from infection through wounds. This disease causes losses of Lawson cypress nursery stock and has been the subject of investigation with the object of isolating the responsible organism.

From the canker on the stem of a small *Chamaecyparis lawsoniana* obtained from a local nursery, there has been isolated what apparently is a species of Monochaetia, believed to be responsible for the production of the canker. By means of inoculation of a number of trees of *Chamaecyparis lawsoniana* with the organism grown in pure culture, definite proof has been obtained that it is parasitic, capable of killing small seedlings outright, and of producing cankers on stems and branches. From the disease thus produced artificially the organism was reisolated. Further work is anticipated to establish definitely the identity of the organism and determine the extent and nature of the damage it is capable of causing.

The Use of Oil-soluble Copper as a Fungicide.—E. R. DE ONG.

It has been found possible to carry minute amounts of copper into the tissue of leaves and twigs by the use of copper resinate dissolved in a specially prepared pine-tar oil known as Palustrex. Such application offers the possibility of placing copper in contact with the mycelium of fungi after penetrating into plant tissue. Copper has been recovered in measurable amounts from the interior of treated twigs where practically no copper was found on the surface after three weeks exposure. This in contrast with a standard Bordeaux, where 83 per cent of metallic copper was recovered from the surface of leaves and twigs three weeks after application, but with the methods followed none could be detected on the interior of either leaf or twig. The copper resinate is supplied in the form of a heavy paste and dissolved in the Palustrex Oil, which had been especially treated to make it safe for foliage applications. Results to date indicate the possibility of control with much smaller amounts of copper applied in this way than for surface applications of such a material as Bordeaux spray.

Downy Mildew of the Hop in California.—C. EMLEN SCOTT and H. EARL THOMAS.

The hop downy mildew, caused by *Pseudoperonospora humuli,* was recognized for the first time in California on May 1, 1934. The wide distribution of the fungus in Sonoma and Mendocino Counties indicates that it is of remote introduction into this State. Local leaf infections, spike formation of terminal growth, and nodal infections as described by earlier workers are present in abundance.

The disease was found in severe form in escaped plants that are, however, seldom found, except in the immediate vicinity of hop yards. No seedling hop plants have been seen.

In 2 distinct infection periods during late May and June, the apparent incubation time for leaf spots was 7 to 9 days, and 9 to 11 days for the production of spikes. These periods were marked by rains preceded and followed by relatively dry weather.

There is some evidence to indicate that considerable resistance is developed by stems with increasing age.

Control of Soil Fungi by Fumigation with Chloropicrin.—G. H. GODFREY.

Fumigation tests were conducted with chloropicrin against various plant-parasitic soil fungi, including *Fusarium* sp. from gladiolus, *Verticillium albo-atrum* from strawberry, *Phytophthora cactorum* from snapdragon, *Rhizoctonia solani* and *Sclerotium rolfsii* from sugar beet, and the root rotting fungi *Armillaria mellea* and *Dematophora* sp. Four-gallon glazed stone jars, filled with autoclaved greenhouse soil, were used. Cultures of the fungi were introduced in small glass cells, containing slightly moistened soil wrapped with cheesecloth. These were thrust into the soil adjacent to the sides of the jars and about half way down. There they were allowed to incubate for 24 hours. Chloropicrin was inserted to a depth of 3 inches in the center of each jar, at the rate of

1¼ cubic centimeters per jar (equivalent to 400 pounds per acre-foot). The jars were sealed with a glue-coated paper cover. The cultures were removed after 48 hours and plated on agar. All fumigated cultures were completely negative for growth. All control cultures, treated identically, except for the gas, gave good growth.

New Economic Hosts of the Stem and Bulb Nematode.—G. H. GODFREY and C. EMLEN SCOTT.

Salsify, *Tragopogon porrifolius;* parsley, *Petroselinum hortense;* and celery, *Apium graveolens,* are reported as hitherto unrecorded hosts of the stem- and bulb-infesting nematode *Anguillulina* (Tylenchus) *dipsaci.* Diseased salsify plants, submitted for diagnosis in the spring of 1933, from San Juan, San Benito County, California, were found infested by this nematode. Symptoms in salsify were swollen leaf bases and "tulip-root" effect in young shoots, with some necrosis in the crown region. Failing near-by parsley plants of the double and triple-curled varieties and Hamburg, a fleshy-rooted variety, all showed typical symptoms of stem-nematode infestation. Symptoms were dwarfing of leaves and leaflets, tulip-root effect with occasional cracking in leaf petiole bases, and occasional rotting off of leaves at the crown. Nematodes identified as *A. dipsaci* were found in petioles and leaflets. In addition there was found extremely heavy infestation within the fleshy roots. This constitutes a new type of injury caused by this organism. The salsify and parsley followed a crop of garlic, *Allium sativum,* known to have been heavily infested with the stem nematode.

The third new host, celery, was not found in the field, but as a result of greenhouse inoculation with colonies of nematodes from garlic and parsley.

The Use of Carborundum as an Abrasive in Plant-Virus Inoculations.—T. E. RAWLINS and C. M. TOMPKINS.

Spotted wilt of tomato, *Lycopersicum esculentum,* and lettuce, *Lactuca sativa* L., virus diseases of cauliflower, *Brassica oleracea* L., var. *botrytis* L., broad bean, *Vicia faba* L. var. *minor,* and stock, *Mathiola incana* R. Br., are difficult to transmit by the ordinary methods of mechanical inoculation. These diseases may be readily transmitted by the use of the following technic. Powdered carborundum (370 grain), contained in a salt shaker, is dusted over the upper surface of the leaves to be inoculated. A piece of absorbent cotton, held in forceps, is dipped in virus that has just been extracted from young plants showing the first symptoms of the diseases. The virus-soaked cotton is then rubbed lightly over the carborundum-coated leaves. It is supposed that the sharp particles of carborundum penetrate the epidermal cells without killing them and allow the virus to enter.

Soil Treatments for the Control of Cotton Root Rot Caused by Phymatotrichum omnivorum.—R. B. STREETS.

One of the most serious aspects of the whole root-rot problem is the destruction of many valuable long-lived perennials, such as deciduous fruit trees, nut trees, and ornamental trees and shrubs. Our results indicate that heavy applications of ammonium sulphate or ammonium hydrate will prove successful in checking root rot in some tree crops. Outstanding success has resulted from treatment of pecan trees in the Yuma valley. In one orchard no treated trees were lost; in the second grove about 40 per cent of the treated trees died while there was a 100 per cent loss of nontreated trees. Experiments on deciduous fruit trees have been complicated by the great variability of soils in the orchards treated, but results have been mainly encouraging. The use of organic manures, either of high or low nitrogen content, in combination with the above

treatments appears promising. Since both ammonium compounds, especially the hydrate, are very toxic to the trees if applied in too great concentration, a special method was evolved for safe and accurate application under field conditions. While experiments have been conducted largely with badly diseased trees, it is obvious that the effect of treatment would be much more valuable if applied to trees only slightly affected or standing in the path of the advancing fungus. Such trees might be maintained without loss of production, while badly diseased trees must usually be severely pruned, entailing a loss of one or more crops.

Some Factors Influencing the Sporulation of Certain Species of Phytophthora.—L. J. KLOTZ and H. S. FAWCETT.

Mycelium of *Phytophthora citrophthora, P. parasitica,* and *P. cactorum,* grown in prune extract, produce abundant, normal sporangia when transferred to and kept in a shallow layer of water permitting some of the hyphae to protrude above the water level.

Cleavage of sporangial protoplasm and discharge of sporangia are then readily induced by exposure to two temperatures, 28°–30° C. for 3 to 5 minutes, followed by 15°–18° C. for 1 hour. Swarming begins in a few minutes and reaches a maximum in approximately an hour under these conditions. With *Phytophthora citrophthora,* exposure only to light (artificial or sunlight), or to smaller depressions in temperature, as from 28° to 23° C., in either light or darkness, also stimulate swarming, but the above procedure consistently yields abundant zoospores.

Zoosporangia of *Phytophthora citrophthora* from mycelium grown in glucose potato broth differ from those developed on mycelium from prune extract. In general, they are smaller, fewer in number, form more slowly (first evidence after 5 or 6 days at 20°–22° C., whereas those from prune juice appear as early as 36 hours after transfer to water) and they divide into zoospores less readily, frequently discharging the protoplasm abortively by plasmoptysis.

Mycelium from both media, used full strength (prune extract: 60 gm. pulp per liter; glucose potato broth: 20 gm. dextrose and 250 gm. sliced potatoes per liter) was more abundant and grew more sporangia than mycelium from the media diluted to ½ concentration. Cultures grown 1, 2, and 4 weeks, respectively, produced similar numbers of zoosporangia when transferred to water.

Sporangia appeared earlier on mycelium from acid media (pH 4.08–pH 6.25) than that from alkaline media (pH 7.9–8.4), although the ultimate numbers were similar, the fungus changing the reaction of both alkaline and acid sets to the acid range, pH 4.16–pH 4.92.

Physiologic Specialization in Phytophthora Species.—F. P. MEHRLICH.

From experiments to determine the relationship of diseases of other economic plants to pineapple heart rot, evidence was obtained that was indicative of physiologic specialization in species of Phytophthora. The cultures tested were separated into 3 categories: (–) incapable of causing heart rot, (+) slightly pathogenic, and (++) virulently pathogenic to the pineapple plant. These are listed below by species, source and number of cultures tested: *P. cambivora* from chestnut 2 cultures –, host unknown 4–; *P. cinnamomi* from cinnamon (cultures of parallel history) 1–, 1+, 1++; from pineapple (Australia) 1++, (Hawaii) 7++; from Rhododendron 1+; from chestnut (S. Europe) 1–; walnut (Australia) 1–; *P. palmivora* (*P. arecae*) host unknown 1–, 2+; (*P. faberi*) host unknown 2–, from coconut 1+; (*P. meadii*) from Hevea 1++, pineapple 1++, (Cacao group) from papaya 1–; Ricinus 1–, coconut 1–, Palmyra 1–, host unknown 1++; (Rubber group) from coconut 2–, 1+, Hevea 1+; *P. parasitica* (*P. melongenae*) from egg

plant 1++, Antirrhinum 1++; from potato 2++, tomato 1++, rhubarb 1+, host unknown 2++.

These data support similar findings by Leonian, Tucker, and Ashby, who used other species and other hosts. They cast considerable doubt on the validity of separating species on the basis of pathogenicity. Such a separation of *Phytophthora cambivora* and *P. cinnamomi*, made on the basis of the differential susceptibility of potato tubers to their attack, seems highly questionable.

Some Host Responses in Graft Transmissions of Dieback Streak of Tomatoes.—MICHAEL SHAPOVALOV.

Mechanical transmission of this form of streak was obtained with *Nicotiana tabacum* and *N. glutinosa* in addition to tomato and *Datura stramonium*, as previously reported. *N. glauca* gave no definite results; a faint leaf mottling or a few necrotic spots appeared in some cases but the virus was not recovered. All these hosts were used also in grafting experiments. All grafts were made by the cion method, using diseased cions for healthy stocks and healthy cions for diseased stocks. Successful unions, as a rule, brought about the transmission of the disease to susceptible hosts. Symptomatological responses in such cases were similar to those obtained in the juice transmission. Graft-inoculated tomato, tobacco and *N. glutinosa* developed severe streak necrosis. Healthy Datura grafted with either of these diseased plants developed blotchy, puckering, coarsely-mosaic leaves and no necrosis. These mosaic Datura plants grafted with healthy tomato, tobacco, and *N. glutinosa* brought about the development of typical dieback streak in these susceptible hosts. Healthy *N. glauca* grafted with streaked tomato, tobacco, and *N. glutinosa* plants occasionally developed scarce circular necrotic spots on older leaves, but otherwise remained symptomless. Tops of such *N. glauca* plants grafted with healthy susceptible plants transmitted no disease.

VI. INTERNATIONAL BOTANICAL CONGRESS

Amsterdam, September 2–7, 1935

The Organizing Committee of the VI. International Botanical Congress announces that the following topics preliminarily have been chosen for discussion in the sections:

AGR. Agronomy. 1). Interactions between roots and soil; interactions between plants. 2). Virus diseases. 3a). Weed flora as an indicator of soil conditions in agriculture. 3b). Grassland associations. 4a). Genetics and breeding of immune varieties. 4b). Inbreeding. 5). Importance of microbiological investigations in the study of agricultural problems. 6). Influencing the cycle of development in plants.

CYT. Cytology. 1). Structure of chromosomes. 2a). Crossing-over versus conversion. 2b). Terminology of cytology and genetics. 3a). Pairing of chromosomes in polyploids. 3b). Reduction division in fungi. 4). Chain- and ringformation of chromosomes. 5a). Submicroscopic structure of the cell wall. 5b). Vacuome, chondriome, plastids. 6). Colloid chemistry of protoplasm; vital staining.

GEN. Genetics. 1a). Experimental mutations. 1b). Genetical basis of size and form. 2a). Crossing-over versus conversion. 2b). Terminology of cytology and genetics. 3a). Sexuality in fungi. 3b). Reduction division in fungi. 4a). Genetics and breeding of immune varieties. 4b). Inbreeding. 5). Taxonomy and genetics. 6a). Plasm and genotype in their mutual relations. 6b). Lethal factors.

GEO. Geobotany, ecology and phytogeography. 1). Climax associations in northwestern Europe and North America. 2). Cartography: a). Vegetation maps; b). Area maps. 3). Flora and vegetation area. 4). Plant geography in younger formations. 5). The halophyte problem. 6a). Classification and nomenclature of vegetation units. 6b). Miscellaneous papers.

MOR. Morphology and anatomy. 1a.). Size and form. 1b). Genetical basis of size and form. 2a). Phytohormones; general paper. 2b). Leaf arrangements. 3). Flower morphology. 4). Female fructification and phylogeny of Conifers. 5a). Wood anatomy. 5b). Relations between anatomy and external morphology. 6). Morphology of bryophytes.

MYC. Mycology and bacteriology. 1). Differential characters in Hymenomycetes. 2). Nomenclature of fungi. 3a). Sexuality in fungi. 3b). Reduction division in fungi. 4). Biologic forms of fungi. 5). Importance of microbiological investigations in the study of agricultural problems. 6). Phylogeny and taxonomy of Phycomycetes.

PATH. Phytopathology. 1). Biological basis of plant quarantine. 2). Virus diseases. 3). Various papers. 4). Biologic forms of fungi. 5). Immunization. 6). Physiologic diseases.

PB. Palaeobotany. 1). Geobotanical provinces in the older formations. 2). Caytoniales and Pteridospermae and the evolution of angiosperms. 3). Flower morphology. 4). Plant geography in younger formations. 5). Synchronism and uniformity in palaeozoic and mesozoic floras. 6). Various papers.

PH. Plant physiology. 1). Photosynthesis. 2a). Phytohormones; general paper. 2b). Phytohormones; various papers. 3). Oxidation, reduction and metabolism. 4). Permeability and the accumulation of mineral elements. 5a). Submicroscopic structure of the cell wall. 5b). Translocation of plastic materials. 6). Influencing the cycle of development in plants.

SYS. Taxonomy and nomenclature. 1). Various papers. 2). Caytoniales and Pteridospermae and the evolution of angiosperms. 3). Flower morphology. 4). Female fructification and phylogeny of conifers. 5). Taxonomy and genetics. 6). Phylogeny and taxonomy of Phycomycetes.